

**A STUDY OF CHANGES IN HISTOLOGY, SEXUAL  
FUNCTION, HAEMATOLOGICAL AND NUTRITIONAL  
STATUS FOLLOWING RESTORATIVE  
PROCTOCOLECTOMY**

**By**

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## **DECLARATION OF ORIGINALITY**

I hereby declare that the work embodied in this thesis is the result of my own independent investigating. This work has not already been accepted for any degree and is not currently being submitted in candidature for any other degree.

**Dominic B. Coull**

For Nadine, Bruce and Elizabeth, whose love and support  
I am immeasurably grateful for.



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## ABSTRACT

Restorative proctocolectomy (RP) has become the gold-standard surgical procedure for Ulcerative Colitis (UC) and Familial Adenomatous Polyposis (FAP). Following proctocolectomy a 1-2cm “cuff” of columnar rectal epithelium is retained that has unknown risk of malignant change. Markers of malignant potential in UC include aberrant p53 expression and dysplasia. We undertook a prospective study comprising serial surveillance biopsy and assessed the occurrence of aberrant p53 expression, epithelial dysplasia and carcinoma in the retained anorectal cuff following RP.

RP is often performed on young adults within the peak parenting age bracket. Pelvic dissection may result in damage to the autonomic nerves of sexual function and consequently affect fertility in male and female patients. We examined the impact of ileo-anal pouch formation on male and female sexual and reproductive function.

RP involves terminal ileal resection and formation of a small bowel reservoir that encourages bacterial overgrowth. These factors may predispose to nutritional deficiencies, especially low vitamin B12 levels. We examined short and long-term haematological and nutritional status following RP.

No dysplasia was found within cuff-surveillance biopsies up to 12 years post-RP, suggesting that cuff surveillance in the first decade after pouch formation is unnecessary, but should continue for patients with high grade dysplasia or carcinoma in the original colectomy specimen. p53 expression was not useful in pre-dysplastic surveillance of cuff biopsies from patients who have undergone RP for UC.

One quarter of our patients developed a subnormal vitamin B12 levels following RP. The cause of this is uncertain. Serum B12 levels should be measured during follow up and those with subnormal B12 levels, should see them successfully restored to a normal value after treatment with oral B12 replacement therapy. On both short and long term follow-up, the majority of patients maintained a normal micronutrient profile.

Pregnancy rates decreased following RP and this was associated with a high incidence of male and female sexual dysfunction.

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## **ABBREVIATIONS**

BIA	Bio-electrical Impedance Analysis
FAP	Familial Adenomatous Polyposis
HGD	High Grade Dysplasia
IBD	Inflammatory Bowel Disease
IRA	Ileo Rectal Anastomosis
LGD	Low Grade Dysplasia
PSC	Primary Sclerosing Cholangitis
RP	Restorative Proctocolectomy
STC	Slow Transit Constipation
UC	Ulcerative Colitis

## **APPENDICES**

- Appendix i Letter accompanying sexual/reproductive function questionnaire
- Appendix iii Female sexual/reproductive function questionnaire
- Appendix iv Male sexual/reproductive function questionnaire



## **PUBLICATIONS**

- 1) *Authors' reply: Vitamin B12 deficiency following restorative proctocolectomy*  
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- 2) *Vitamin B12 deficiency following restorative proctocolectomy*  
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- 3) *Long term cancer risk of the anorectal cuff following restorative proctocolectomy assessed by p53 expression and cuff dysplasia.*  
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- 4) *Histological assessment of the columnar cuff after stapled restorative Proctocolectomy.*  
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## **PRESENTATIONS**

### **VERBAL**

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**DB Coull**, JH Anderson, RF McKee & IG Finlay  
Travelling Surgical Society, Ashford, Middlesex. September 2006
- 2) *Plasma homocysteine levels indicate vitamin B12 deficiency is common following restorative proctocolectomy.*  
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- 3) *Oral Cyanocobalamin Corrects Commonly Occurring Vitamin B12 Deficiency Following Restorative Proctocolectomy - Should All Pouch Patients Be Prescribed Oral B12?*  
**DB Coull**, JH Anderson, RF McKee & IG Finlay  
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- 4) *Pregnancy Rates fall Dramatically after Male and Female Restorative Proctocolectomy.*  
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International Tripartite Association of Coloproctology of GB, USA and Australia, Melbourne. October 2002
- 5) *Why do at Least One Quarter of Pouch Patients Develop Vitamin B12 Deficiency, and How Should it be Treated?*  
**DB Coull**, JH Anderson, RF McKee & IG Finlay  
American Society of Colon and Rectal Surgeons, San Diego. June 2001

- 6) *Dysplasia & p53 Expression Following Stapled Restorative Proctocolectomy for Ulcerative Colitis*  
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- 7) *At Least One in Four Patients Develop Vitamin B12 Deficiency Following Ileoanal Pouch Formation for Ulcerative Colitis- Is Oral B12 Replacement Sufficient?*  
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- 8) *Vitamin B12 Deficiency after Ileoanal Pouch Formation- What is the Cause?*  
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- 9) *Vitamin B12 Deficiency after Ileoanal Pouch Formation- What is the Cause?*  
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- 10) *The Risk Of Dysplasia in the Residual "Cuff" after Stapled Restorative Proctocolectomy*  
**DB Coull**, FD Lee, JH Anderson, RF McKee, IG Finlay  
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- 11) *Is Pouch Cuff Surveillance for Ulcerative Colitis Necessary in the Short Term?*  
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- 12) *Ileoanal Pouch Cuff Surveillance, Is it Necessary?*  
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- 13) *For Whom the B12 Falls* **DB Coull**, JS McCartney, IG Finlay  
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- 14) *Cuff Surveillance, Duff Surveillance?*  
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- 15) *For Whom the B12 Falls*  
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## POSTER

- 1) *Female Sexual Dysfunction is Common following Restorative Proctocolectomy*  
**DB Coull**, JH Anderson, RF McKee & IG Finlay  
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- 2) *High Incidence of Male Sexual Dysfunction following Restorative Proctocolectomy*  
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International Tripartite Association of Coloproctology of GB, USA and Australia, Melbourne. October 2002
- 3) *Dysplasia & p53 Expression Following Stapled Restorative Proctocolectomy for Ulcerative Colitis*  
**DB Coull**, FD Lee, JH Anderson, RF McKee, IG Finlay  
American Society of Colon and Rectal Surgeons, San Diego. June 2001
- 4) *Serum B12 Falls After Pouch Surgery but Bacterial Overgrowth is not the Cause*  
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BSG, Glasgow, March 2001
- 5) *Ileoanal Pouch Cuff Surveillance, Is it Necessary?*  
**DB Coull**, JS McCourtney, FD Lee, JH Anderson, RF McKee, IG Finlay  
Association of Coloproctology of GB & I, Southport, June 1999

# **CHAPTER ONE**

## **INTRODUCTION**

## **1.1 Restorative Proctocolectomy-Historical Background**

The first description of complete excision of the large bowel with sphincter preservation, was by Ravitch and Sabiston in 1947.<sup>1</sup> Prior to the adoption of creating an ileal reservoir, the 'straight ileo-anal anastomosis' was performed by a few surgeons at the time. The functional results were subsequently reviewed by Valiente and Bacon, who concluded that function was often poor, largely due to the resulting urgency and frequency.

Kock's introduction of the continent ileostomy established that a small bowel reservoir could successfully function in humans.<sup>2</sup> Subsequently, Parks and Nicholls<sup>3</sup> combined this concept with their own endo-anal anastomotic technique, to create the first ileo-anal ileal reservoir in 1978, a technique that is now universally referred to as restorative proctocolectomy (RP).

Due to its complexity, this procedure is associated with a significant morbidity but the mortality is very low. Despite encouraging results since its inception, the extended outcome of RP remains to be seen. Therefore, its long-term future may be described as uncertain but it is now the most frequently employed procedure in the surgical treatment of Ulcerative Colitis (UC).<sup>4</sup>

Although initial reports indicated that the straight ileo-anal procedure could result in acceptable function,<sup>5</sup> there are now data to show that capacitance of the neo-rectum is inversely related to frequency – whether this is straight ileum,<sup>6</sup> ileal reservoir<sup>7</sup> or colonic reservoir.<sup>8</sup>

### 1.1.1 Indications for RP

RP is commonly performed for UC, also for Familial Polyposis Coli (FAP) and less frequently for Slow Transit Constipation (STC).

Any severely ill patient should firstly undergo a colectomy. The sole reason for performing RP is to avoid a permanent ileostomy. A conventional proctocolectomy gives excellent results, but does encompass a permanent stoma. Where a cancer is also present on a background of UC, it must be possible to achieve local clearance to the same extent as when considering anterior resection or total rectal excision for a standard rectal carcinoma. RP should not be performed on patients with disseminated cancer or Crohn's disease. When RP has been performed on patients with Crohn's disease (usually operated on through mistaken diagnosis), complications are common and failure rates over 20% have been reported, where failure is defined as pouch removal and formation of a permanent ileostomy.<sup>9-14 15</sup>

**Table 1** Outcome of RP performed on patients with Crohn's disease

<u>Reference</u>	<u>N</u>	<u>Ileostomy</u>	<u>Failed</u>
Galanduik et al <sup>9</sup>	16	9	4
Deutsch et al <sup>10</sup>	9		4
Hayman et al <sup>11</sup>	25	1	7
Grobler et al <sup>12</sup>	20		8
Panis et al <sup>14</sup>	31		2
Regimbeau et al <sup>15</sup>	41		3
Tulchinsky et al. <sup>13</sup>	13		6

However Panis et al.<sup>16,17</sup> described a failure rate of 10% at 10 years. It may be that some of these had "indeterminate colitis" (they were all selected as having rectal Crohn's disease without any anal involvement).

When failure rates of patients with UC or Crohn's disease undergoing RP, were assessed in a large series with 10 years or more follow-up, they were reported as 10% and 50% respectively.<sup>18;19</sup> To minimise the risk of performing pouch surgery on patients with Crohn's disease, when caring for patients labelled with indeterminate colitis, it is essential to examine the small bowel radiologically and to identify past or present anal disease. RP should not be carried out on patients with active anal disease such as fissure in ano, ulceration or anorectal sepsis. One study reported that the presence of an anal lesion at surgery increases the risk of anastomotic leakage.<sup>20</sup> Carefully selected patients with an unequivocal diagnosis of UC may have a satisfactory result but anal disease is known to increase the risk of pouch-perineal fistulae and subsequent pouch failure.<sup>21</sup>

The integrity of the anal sphincter must be sufficient and preoperative examination may need to be supplemented by anal manometry when there is doubt. Age itself should not be a contraindication. Provided the indications for surgery are satisfied and the patients have given informed consent, the decision rests with them.

Patients with sclerosing cholangitis have two times the incidence of pouchitis after RP.<sup>22</sup> While not a contraindication to undergoing RP, the patient should undergo careful preoperative counselling before deciding to pursue pouch formation. Liver function tests should be part of the routine preoperative assessment.

The personal component is very important, the choice lies between a restorative or conventional proctocolectomy and is almost entirely the patient's choice to make. This decision can only be made if a full explanation is given of the possible complications. These include failure and complication rates, total treatment time, the possibility of pouchitis and the likely functional outcome. A multi-disciplinary

approach including a ‘pouch support’ nurse, stomatherapist, and patient-support group is beneficial.

### **1.1.2 Technique**

Whilst the basic technique has remained the same since its inception in 1978, some modifications have been made, including; the design of the pouch, the method of rectal dissection, the technique of ileo-anal anastomosis and whether a mucosectomy should be carried out. Use of a defunctioning temporary ileostomy is no longer regarded as mandatory.

The operation is performed under general anaesthesia with the patient in the Lloyd-Davis position with the hips and knees each flexed to about 120 degrees.

Several different anatomical types of ileal reservoir have been described. The main design factors to consider are capacitance and emptying characteristics. The original three loop “S” reservoir of Parks has largely been superseded because of the evacuation difficulty that often resulted from the short segment of ileum, distal to the pouch. The two loop “J” reservoir<sup>23</sup> is easy to make by hand or by stapling and its limbs should approach 20cm in length. The four loop “W” reservoir<sup>24;25</sup> achieves a capacitance greater than the J reservoir and similarly has no distal segment.

Because of its simplicity in construction, the ‘J’ design is the most widely used. The ‘W’ design is unarguably more complex. Generally, the larger the capacitance of the reservoir, the lower the frequency. Nicholls & Pezim<sup>7</sup> reported a comparison of frequency of defaecation between ‘S’, ‘J’ and ‘W’ shaped reservoirs. They observed an inverse relationship between the frequency of defaecation and the maximum tolerated volume of the reservoir, as measured by inflation of a balloon introduced into the pouch through the anus. This would suggest that capacity is a factor



determining frequency. The overall impression from studies examining pouch design and frequency is that it does not really matter what configuration of pouch is made as long as it is large enough.<sup>26</sup> It is well recognised that, with time following closure of the ileostomy, pouch volume increases.

A large percentage of patients undergoing RP are young and have benign disease. Consequently, great care is taken to preserve the pelvic autonomic nerves. Damage to these nerves may result in sexual dysfunction and/or urinary problems. In the male, damage to the autonomic nerve plexus, in the lower part of the pelvis on the lateral wall, causes failure of erection (impotence). Damage to the presacral nerves results in failure of ejaculation.

The chance of pelvic nerve injury is reduced by adopting the technique of close rectal dissection<sup>27</sup> in patients without dysplasia and therefore without the risk of harbouring an undiagnosed cancer. This technique involves removal of the rectal tube itself without any surrounding mesorectum. The blood vessels are divided as they enter the rectum. This is regarded as time-consuming and difficult by many surgeons but offers a certain way of preventing pelvic nerve damage.

Most surgeons, utilise the same total mesorectal excision technique as for a cancer dissection, but identify the presacral nerves in the upper part and stay close to the rectal wall in the lower part. For patients who have dysplastic changes confirmed pre-RP, a close rectal dissection should not be carried out since subclinical invasion may have already developed.

The operative steps replicate those of conventional proctocolectomy until the rectum is fully mobilised. Dissection continues down to the level of the anorectal junction where the bowel is divided, following antiseptic washout of the anal canal. The method of division differs, depending upon whether a hand-sewn or stapled

anastomosis is performed. For hand-sewn anastomoses the bowel is divided at a distance of about 2cm above the dentate line. The correct level is indicated by a perineal operator carrying out a simultaneous proctoscopy. For stapled anastomoses, a transverse staple line is placed across the rectum at the anorectal junction. Precision in the correct placement of the staple line is paramount.

Once the proctocolectomy specimen is removed, the small bowel mesentery should be carefully dissected to achieve full mobility. A trial descent to the anal canal level, using the part of the ileum selected for the ileo-anal anastomosis, is recommended. If it does not reach, mesenteric vessels may be divided to offer greater length. They should be carefully selected to avoid ischaemia.

The results from two randomised trials comparing hand-sutured and stapled ileo-anal anastomoses.<sup>6;28</sup> have shown no important difference in morbidity or function. The manual technique permits a precise level for the anastomosis and avoids the possibility of leaving rectal mucosa behind. Although there are techniques available to permit the accurate placement of the transverse stapler within the anal canal,<sup>29</sup> stapling may sometimes result in retention of rectal mucosa. This residual mucosa may be affected by inflammation resulting in bleeding, discomfort and urgency. Even if the anastomosis is carried out at the correct level, the residual columnar epithelium in the anal stump may lend itself to similar symptoms such as bleeding, peri-anal burning and frequent evacuation of small volume stool. 'Strip proctitis' has been reported in around 10-15% of cases having a stapled anastomosis.<sup>12;30</sup> There is furthermore a small cumulative risk of dysplasia of 3% over a mean follow-up of 16 months.<sup>31</sup>

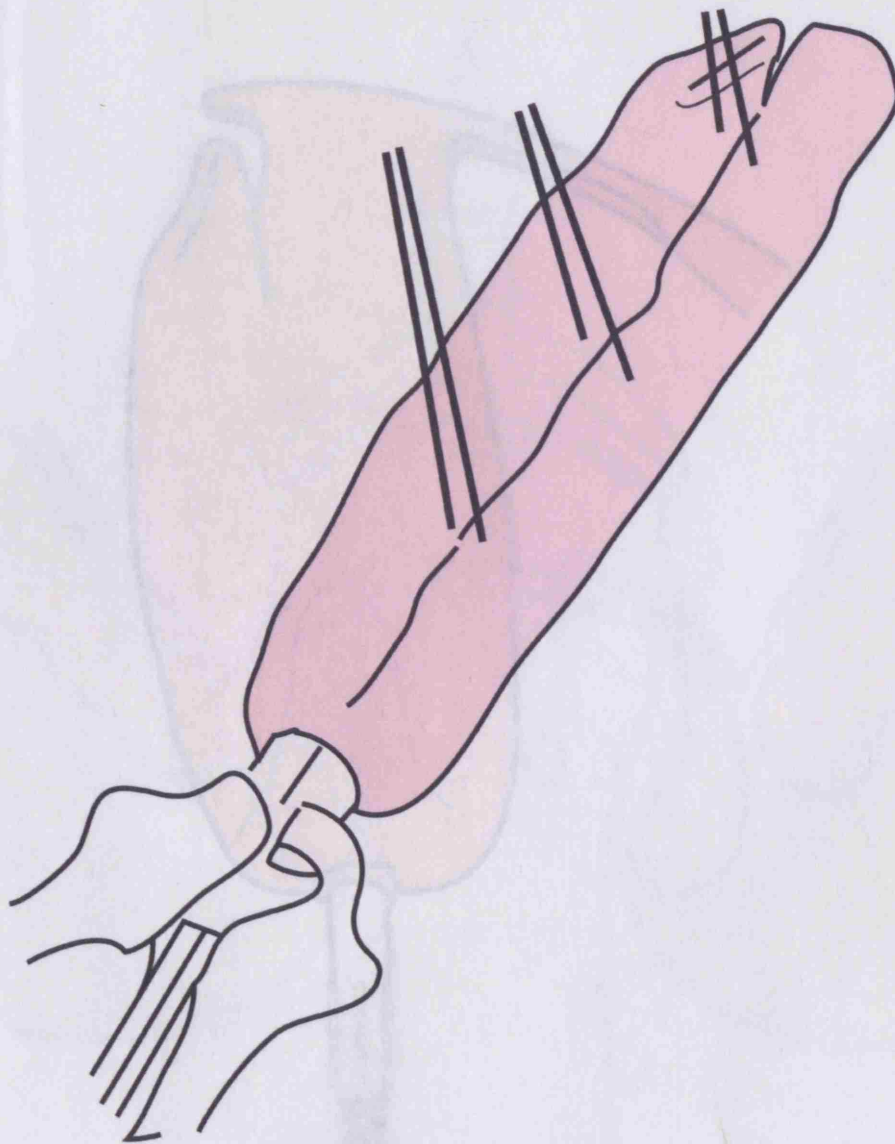
Stapling may be complicated by postoperative anastomotic stenosis, which is less common following a hand-sewn anastomosis. Studies show that up to 40% of pouches

with a stapled anastomosis can stenose and therefore require repeated dilatation or the daily use of anal dilators.<sup>24;32;33</sup>

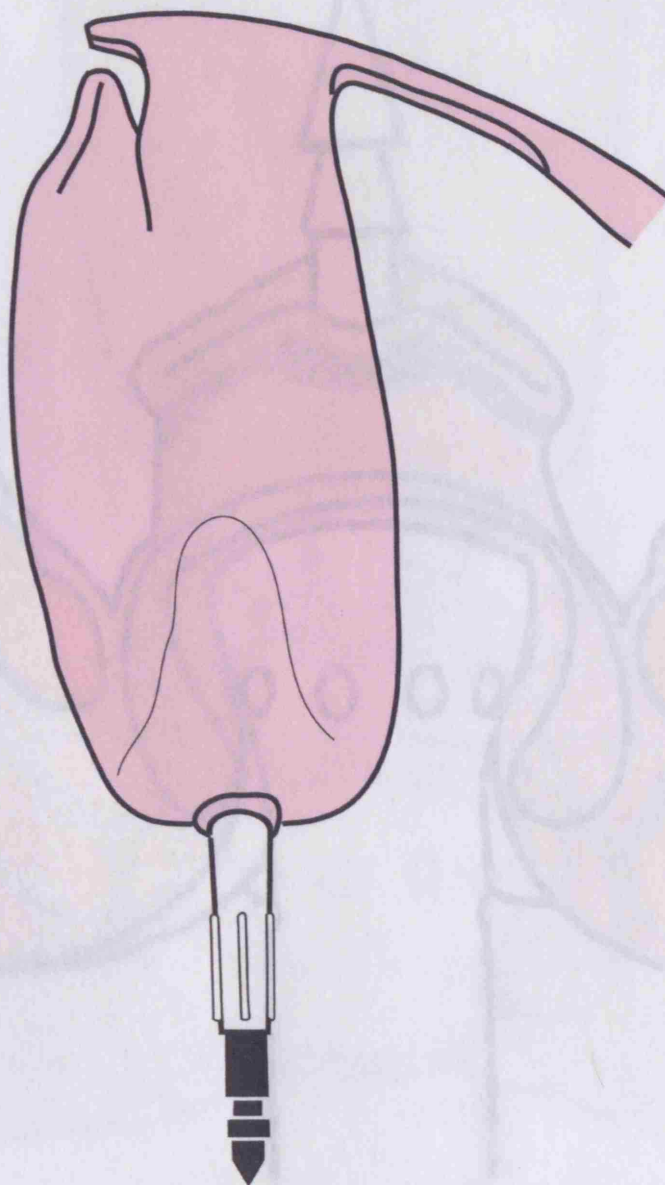
If descent of the small bowel to the anastomotic level might result in excessive tension in the mesentery, a stapled anastomosis at the anorectal junction is preferred.

Both manual and stapled anastomoses are followed by a fall in resting anal canal pressure.<sup>34</sup> This is greater with the former but has not affected continence significantly in clinical trials. The surgeon should be capable of performing either technique.

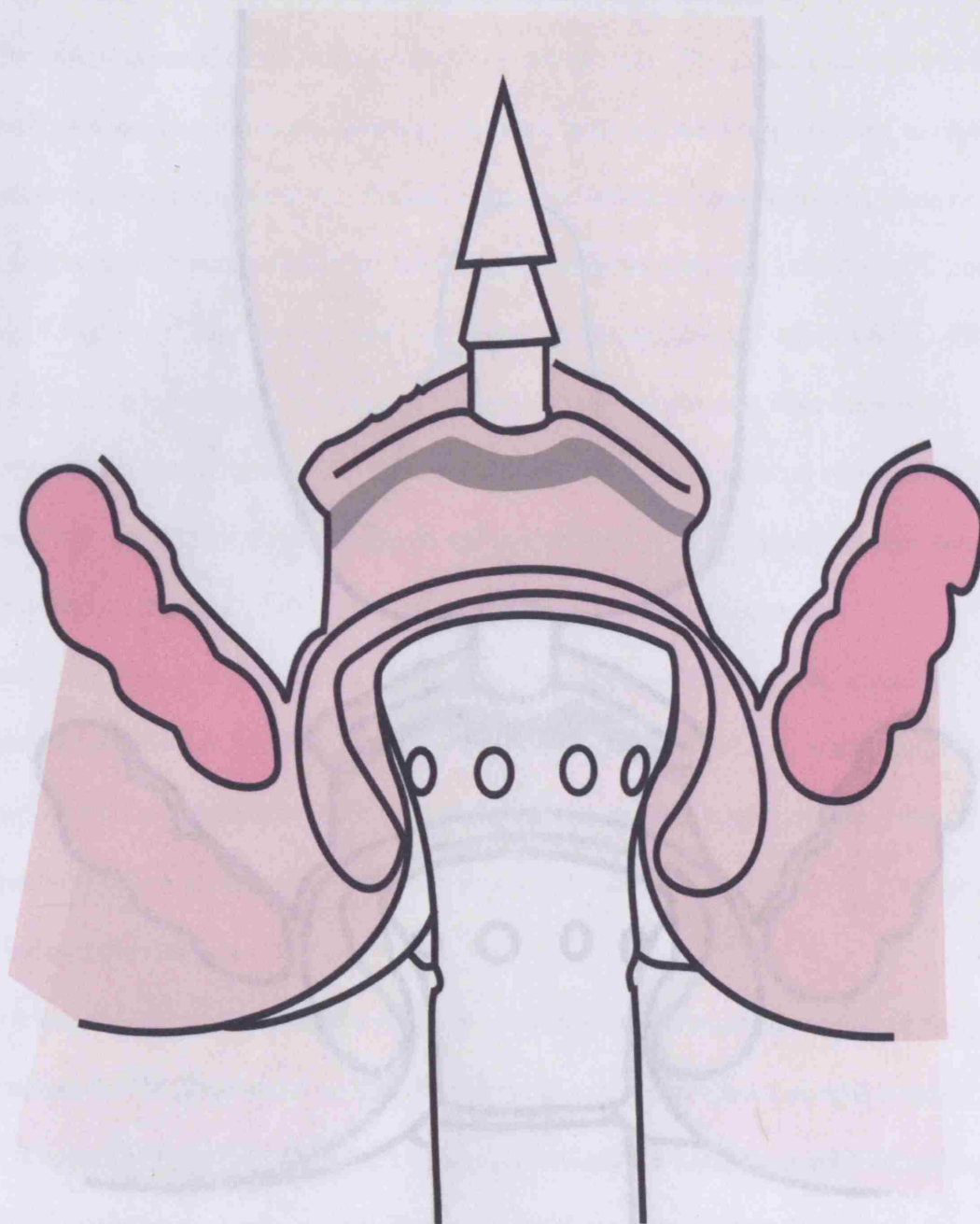
**Figure 1** Creation of a side-to-side ileoileal anastomosis with a long linear cutting stapler to form a long J-pouch



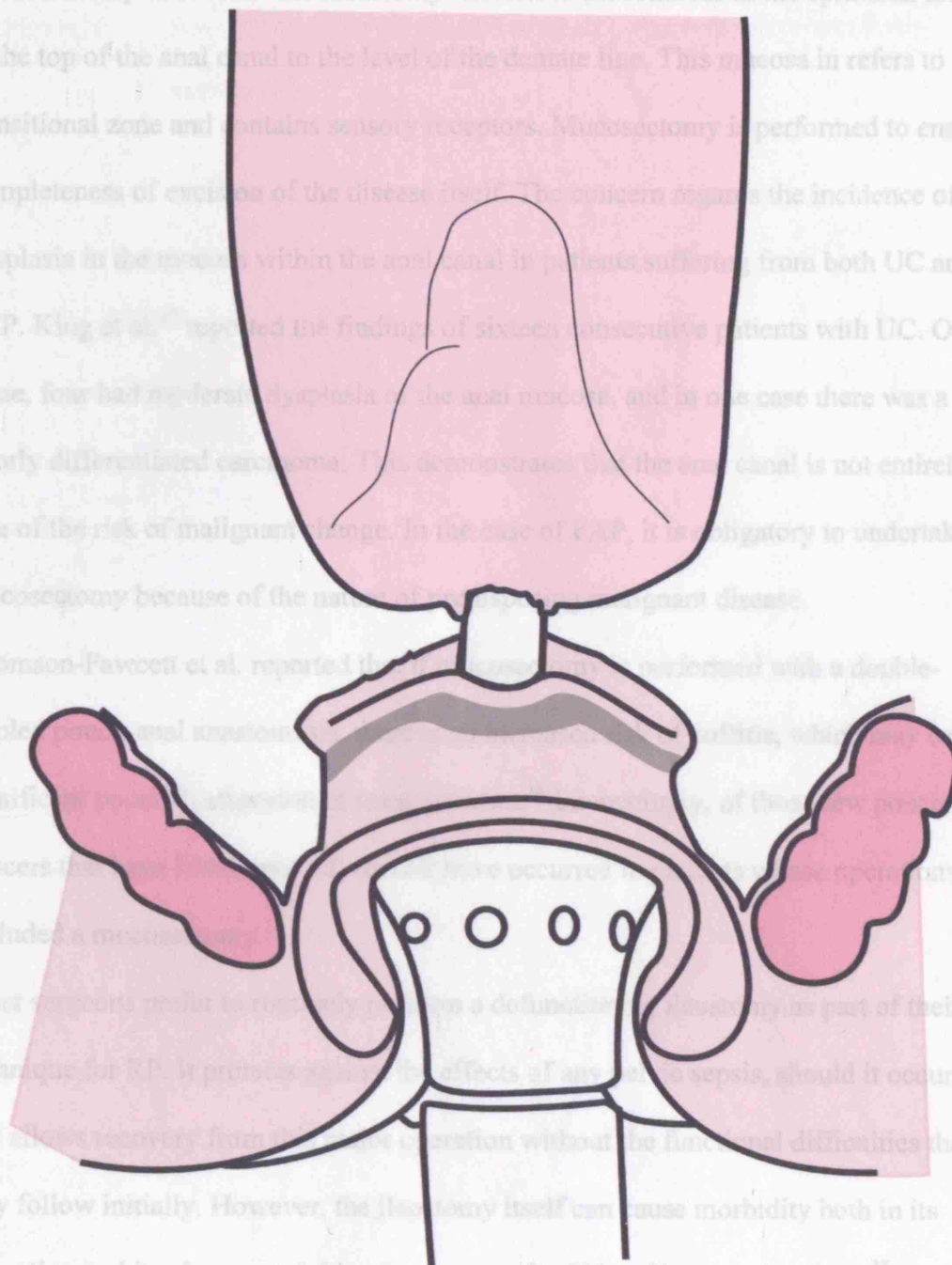
**Figure 2** Double-stapled J pouch – anvil of circular stapling device inserted into the pouch and secured by a purse string around the enterotomy



**Figure 3** Division of the anal canal 1-2cm above the dentate line. Head of circular stapler is inserted into the anal canal followed by advancement of the central pin



**Figure 4** Ileal J pouch-anal anastomosis using circular stapling device



Successful results without ileostomy have been reported<sup>39</sup>. One randomised trial showed that pelvic sepsis was no more frequent in patients without a defunctioning ileostomy.<sup>40</sup> However, 10-20% of patients required ileostomy formation in the immediate post-operative period, sometimes as an emergency. The clinician has to

Whether or not to include a mucosectomy in the operative technique is another area of controversy. The term 'mucosectomy' describes the removal of the epithelial lining at the top of the anal canal to the level of the dentate line. This mucosa in refers to the transitional zone and contains sensory receptors. Mucosectomy is performed to ensure completeness of excision of the disease itself. The concern regards the incidence of dysplasia in the mucosa within the anal canal in patients suffering from both UC and FAP. King et al.<sup>35</sup> reported the findings of sixteen consecutive patients with UC. Of these, four had moderate dysplasia of the anal mucosa, and in one case there was a poorly differentiated carcinoma. This demonstrates that the anal canal is not entirely free of the risk of malignant change. In the case of FAP, it is obligatory to undertake a mucosectomy because of the nature of predisposing malignant disease.

Thomson-Fawcett et al. reported that if mucosectomy is performed with a double-stapled pouch-anal anastomosis, there is an increased risk of cuffitis, which may cause significant pouch dysfunction in some patients.<sup>36</sup> Interestingly, of those few pouch cancers that have been reported, several have occurred in patients whose operations included a mucosectomy.<sup>37</sup>

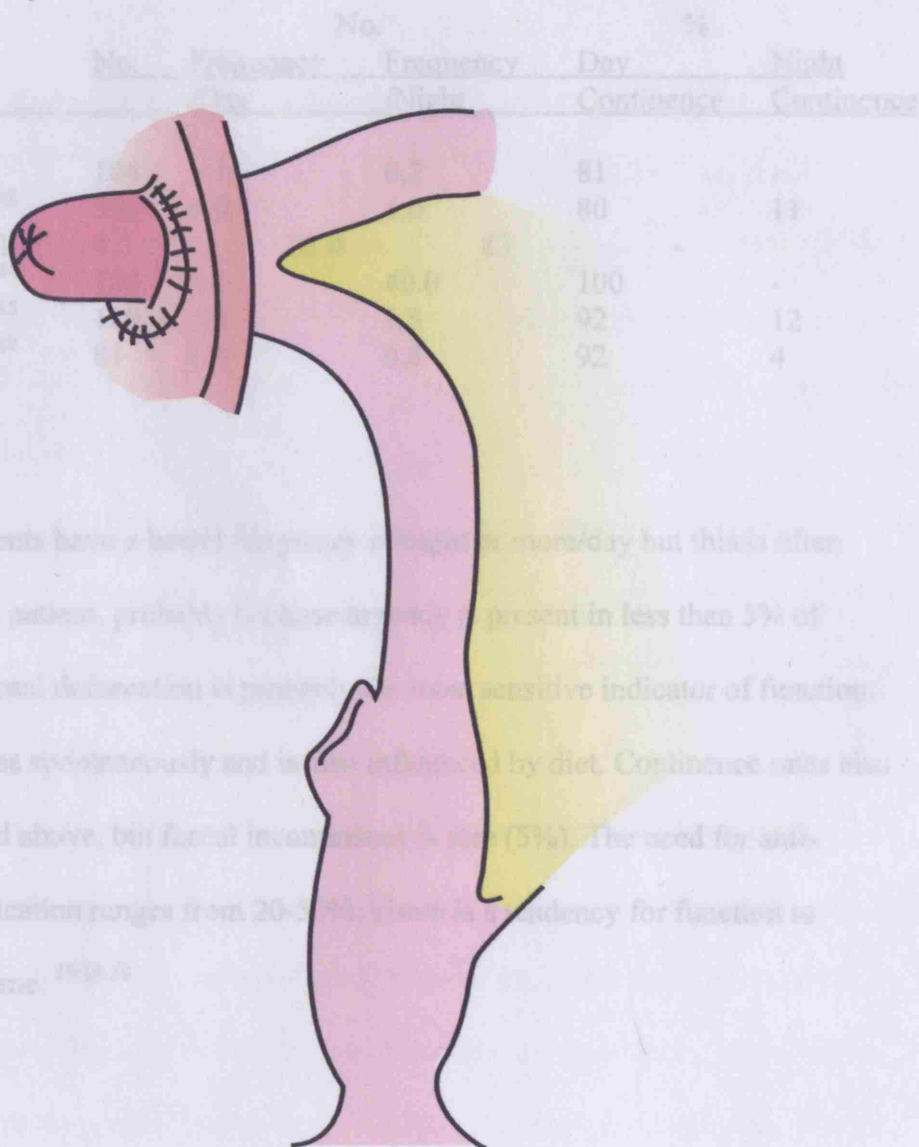
Most surgeons prefer to routinely perform a defunctioning ileostomy as part of their technique for RP. It protects against the effects of any pelvic sepsis, should it occur, and allows recovery from this major operation without the functional difficulties that may follow initially. However, the ileostomy itself can cause morbidity both in its formation and its closure and this may account for 20% of its complications.<sup>38</sup>

Successful results without ileostomy have been reported<sup>39</sup>. One randomised trial showed that pelvic sepsis was no more frequent in patients without a defunctioning ileostomy.<sup>40</sup> However, 10-20% of patients required ileostomy formation in the immediate post-operative period, sometimes as an emergency. The clinician has to



balance a successful 'one stage' procedure in the majority against the potentially serious complications of faecal pelvic sepsis or peritonitis in the minority.

**Figure 5** Stapled J-pouch with defunctioning loop ileostomy to protect low anastomosis



### 1.1.3 Pouch Function

Following pouch surgery, the frequency of bowel evacuation ranges from a median of four, to seven defaecations per day as shown in table 2

**Table 2** Bowel function following RP.

	No.	Frequency	No. Frequency	% Day	Night
		/Day	/Night	Continence	Continence
Schoetz et al. <sup>41</sup>	104	5.1	0.2	81	-
Pemberton et al. <sup>42</sup>	390	6.0	1.0	80	11
Nicholls <sup>43</sup>	141	4.5	22.0	83	-
Oresland et al. <sup>44</sup>	100	5.0	40.0	100	-
Wexner et al. <sup>45</sup>	180	5.4	1.5	92	12
Keighley et al. <sup>29</sup>	81	6.1	0.8	92	4

20-30% of patients have a bowel frequency of eight or more/day but this is often tolerated by the patient, probably because urgency is present in less than 5% of patients. Nocturnal defaecation is probably the most sensitive indicator of function. Frequency varies spontaneously and is also influenced by diet. Continence rates also vary, as detailed above, but faecal incontinence is rare (5%). The need for anti-diarrhoeal medication ranges from 20-50%. There is a tendency for function to improve with time.<sup>25;46-50</sup>

### 1.1.4 Complications

RP is a surgical procedure associated with a high morbidity of 20%-50% of patients.

(**table 3**). Complications include; operative death, pelvic sepsis, pouch failure, anastomotic stenosis and small bowel obstruction.

**Table 3** Morbidity following restorative proctocolectomy

<u>Reference</u>	<u>N</u>	<u>Operative death(%)</u>	<u>Pelvic sepsis(%)</u>	<u>Stenosis(%)</u>	<u>Intestinal obstruction (total/reoperated)</u>
Schoetz et al. <sup>41</sup>	104	0	11(11)	3(3)	25/21
Pemberton et al. <sup>42</sup>	390	1	10(3)	-	
Nicholls <sup>43</sup>	152	1	26(17)	-	?/19
Oresland et al. <sup>44</sup>	100	0	10(10)	4(4)	6/6
Wexner et al. <sup>45</sup>	180	-	7(4)	-	
Keighley et al. <sup>29</sup>	168	0	21(12.5)	25(15)	31/14

Pelvic sepsis is usually due to breakdown of the ileo-anal anastomosis or to an infected haematoma or both. Individual surgeon's definitions of pelvic sepsis vary and this may account for the wide variance of its reported incidence from less than 5% to 20%. Usually, the patient becomes febrile within a few days and rectal examination may reveal an anastomotic defect or extra-luminal induration, indicating the presence of haematoma. Passage of fresh blood is highly significant. An examination under anaesthetic should be performed and any collection drained into the lumen. Stricture of the anastomosis requiring active intervention (either dilatation or a more major procedure) is common.

5-20% of patients develop small bowel obstruction at some stage following RP but most cases resolve spontaneously and further surgery is not usually necessary.

Pouch-vaginal fistula or pouch-perineal fistula in males, is seen in 5-10% of (female) patients and is an important cause of late pouch failure and one large study reported its frequency at 7.5%.<sup>51</sup> This complication may occur long after closure of the ileostomy and Groom et al.<sup>52</sup> found the median interval to be eight months. Patients with indeterminate colitis or Crohn's disease have significantly worse outcomes in comparison with UC patients (hazard ratio 1.4, 2.2 respectively).<sup>51</sup> Prior anal pathology has also been associated with the development of pouch-vaginal fistula.

The presence of perianal abscess or fistula-in-ano, before the construction of the ileal pouch reservoir is associated with 3.7- and 6-fold increases in the risk of developing pouch-vaginal fistula. Defunctioning ileostomy formation is usually deemed necessary, combined with local repair. Success rates using a transvaginal approach are around 60%.<sup>38</sup>

### 1.1.5 Pouch Failure

Failure is defined as the need to remove the pouch and establish a permanent ileostomy. Some authors include patients who are defunctioned without any prospect of having the ileostomy closed. Initial studies reported failure rates of 5-15% during the first twelve months after ileostomy closure as shown in table 4.

**Table 4** Pouch failure following restorative proctocolectomy

<b>Study</b>	<b>N</b>	<b>Follow-up Months (mean)</b>	<b>Overall failure (%)</b>
Gemlo et al. <sup>53</sup>	253	>12	9.9
Foley et al. <sup>54</sup>	460	-	3.5
MacRae et al. <sup>55</sup>	551	>30	10.5
Korsgen <sup>34</sup>	180	>24	17.2
Meagher et al. <sup>56</sup>	1310	24-180(77)	10
Tulchinsky et al. <sup>13</sup>	634	36-288(85)	9.7
Fazio et al. <sup>57</sup>	1975	1-228(49)	4.1

More recent reports show that pouch failure continues to occur over 15 years or more at much the same rate.<sup>18</sup> Causative factors include pelvic sepsis (50%), poor function (30%) and pouchitis (10%). Pelvic sepsis in the early post-operative period confers a threefold increase in the chance of subsequent failure<sup>58</sup> and about 30% of patients who develop pelvic sepsis ultimately fail. Pouch failures may occur well beyond this period, but at a reduced frequency. The reasons include poor function, delayed sepsis in the form of pouch-vaginal or pouch-perineal fistula and occasionally pouchitis. Removal of the pouch has a significant morbidity with delayed healing of the perineal wound in 30% of patients.<sup>59</sup>

### 1.1.6 Long term results

It is widely accepted amongst colorectal surgeons who perform RP, that roughly 10% of pouches will be removed and another 10% of patients will experience significant problems following surgery, such as pouchitis. The remaining four out of every five patients appear very satisfied with their outcomes.

Few studies have examined haematinics following RP, but those that have, reported deficiencies of iron and vitamin B12 in less than 10%, over a follow up period of 2-3 years, that may be associated with anaemia.<sup>60,61</sup>

Following pouch formation the small bowel mucosa alters. Villous atrophy occurs in almost all cases (including those with FAP). This is associated with a dramatic rise in the concentration of bacteria in the pouch increasing from  $10^4 - 10^6$  colony-forming units/g of faeces in the normal terminal ileum  $10^{10} - 10^{12}$  in the pouch with a predominance of anaerobes.

“Pouchitis”- acute inflammation of the pouch will affect some patients postoperatively and is associated with symptoms of urgency, frequency, liquid stool and extra-alimentary manifestations. The aetiology is unknown. It does occur in patients who underwent RP for UC but is exceedingly rare in FAP.<sup>62</sup> It is reported as being more common in those with sclerosing cholangitis<sup>22</sup> and in non-smokers.<sup>32</sup> No specific microbiological aetiology has been found but the favourable response to antibiotics and probiotics suggests a bacterial cause in susceptible individuals. This has been demonstrated objectively in vivo by polymorphonuclear leucocyte scanning.<sup>63</sup>

The diagnosis of pouchitis can only be made on a combination of clinical, endoscopic and histopathological features; the last is essential and must show active inflammation. The natural history is similar to UC with some cases showing an acute

relapsing and others a chronic persisting pattern. Grading systems have evolved to assess severity of pouchitis.<sup>62;64;65</sup> There is evidence that pouchitis occurs early after closure of ileostomy.<sup>11;66</sup> Marked acute inflammation over several years may predispose to dysplasia. One study found dysplastic changes in biopsies taken from 3 of 87 pouch patients, followed for a median of 6 years.<sup>67</sup> Others have found a lower incidence over 12 or more years of follow-up<sup>68</sup>. Dysplasia therefore appears to be rare, but continued endoscopic surveillance with multiple biopsies, at 5-year intervals, is recommended.<sup>69</sup>

Pouchitis treatment consists of antibiotics or anti-inflammatory therapy, and more recently, probiotics. Antibiotics including metronidazole, ciprofloxacin and augmentin, can induce a response in over 80% of patients.<sup>38</sup> If oral metronidazole fails, topical administration may be successful. Persistent use of metronidazole should be avoided due to the possible side effect of peripheral neuropathy. The role of 5-aminosalicylic acid drugs and steroids is uncertain. Xanthine oxidase inhibitors (allopurinol) and short-chain fatty acids have been tried without convincing benefit. Maintenance of remission by daily administration of the probiotic VSL3 has been shown to be effective in 85% of patients compared with controls(0%) when taken over a nine month period.<sup>70;71</sup> However withdrawal is followed by recurrence. Probiotics also appear to be useful when given as prophylaxis.<sup>72</sup>

Only 1-2% of pouch patients require removal of the reservoir for pouchitis. The formation of a defunctioning ileostomy in this situation does not affect the degree of inflammation and excision of the pouch with construction of a new reservoir is again followed by pouchitis. There is evidence that pouchitis occurs early after closure of the ileostomy.<sup>73;74</sup>

Inflammation of the columnar cuff above the anal transitional zone - "Cuffitis," is a cause of pouch dysfunction following stapled RP.<sup>30</sup> In a study of 113 patients, 13% were affected by cuffitis that required treatment.

Marked acute inflammation over several years may be associated with cuff dysplasia.<sup>75</sup> Reports of cuff dysplasia are rare but there are now several reports of carcinoma associated with restorative proctocolectomy. This may be due to pre-existing cancer in the rectum<sup>76</sup> or to carcinoma in the residual distal large bowel mucosa below the ileoanal anastomosis.<sup>77</sup> Carcinoma in the pouch itself has been reported in a Kock reservoir<sup>69</sup> and in five patients after RP.<sup>78</sup> Long-term follow-up is therefore essential.



### **1.1.7. Dietary aspects of ileo-anal pouches**

Since the pouch is fashioned from terminal ileum, this has implications for the absorption of fluid, electrolytes and nutrients and in turn for the maintenance of an adequate nutritional status. In particular, vitamin B12 and bile salts are absorbed in the terminal ileum.

Additionally, the nutritional status of people approaching pouch surgery depends on their general health and underlying condition. Some will require nutritional support preoperatively, and all will require advice with the re-introduction of food post-operatively.

Patients with functional pouches can manage a varied and healthy diet. Some associate changes in pouch function with dietary variations and may restrict their diet to control pouch function, although most find their stool frequency acceptable. There are few published data on the relationship between diet and pouch function to support dietary recommendations but foods suggested to increase and decrease pouch function are listed below.<sup>79</sup>

Foods that thicken stools:	Bananas
	Rice
	Bread
	Potatoes
	Creamy peanut butter
	Marshmallows
	Cheese
	Tapioca
	Yoghurt
	Pasta
	Pretzels

Foods that loosen stools:	Chocolate
	Raw fruits
	Raw vegetables
	Highly spiced foods
	Fried foods
	Greasy foods
	Sugary foods
	Leafy green vegetables

In the jejunum, water and sodium are either absorbed from or secreted into the lumen to maintain the osmolarity of the intraluminal contents at 300 mosm/l and the sodium concentration at 90 mmol/l. Sodium absorption is also linked to the absorption of nutrients, in particular glucose.

In contrast, in the ileum, water and sodium are absorbed, with the absorption of sodium taking place against a concentration gradient and without any linkage to glucose.

Most nutrients, including protein, carbohydrates, vitamins and minerals, are absorbed in the first 200cm of the jejunum. Fat is absorbed throughout the jejunum and ileum, whereas bile acids and vitamin B12 are absorbed specifically in the terminal ileum.<sup>80</sup> Studies of the effect of internal pouch formation on nutrient absorption have focused mainly on water, sodium, bile acids and vitamin B12.

Proctocolectomy results in loss of the colon and its properties of absorbing water and sodium. The ileal pouch is fashioned from 30-60cm of terminal ileum and subsequently undertakes the role of faecal reservoir to reduce bowel frequency.

Following RP, the ileal mucosa of the pouch undergoes gradual transformation to a more colonic-type mucosa exhibiting features of; partial villous atrophy, chronic inflammation, crypt hyperplasia and colonic metaplasia.<sup>81;82</sup> The microflora of the pouch increases to become intermediate between that of normal faeces and that of ileostomy effluent, with implications for the bacterial degradation of bile acids and vitamin B12.<sup>83</sup>

Malabsorption and/or malnutrition are rarely seen following RP<sup>84</sup>. The majority of pouch patients are in good health, maintaining or increasing their body weight.<sup>85;86</sup>

Patients with an ileal reservoir open their pouch on average 3-7 times a day, passing a daily average of 650g of stool of a semi-solid, mushy or liquid consistency.<sup>61</sup> They

maintain normal body stores of water and salt by compensating for the higher faecal losses through renal conservation of sodium and water.<sup>87</sup> This compensatory mechanism increases the incidence of renal calculi.<sup>88</sup> Some of the lost colonic function may be compensated for by an increased absorption of water and salt in the ileum.<sup>84</sup>

Several studies have reported malabsorption of bile acids following RP<sup>82;84;89</sup> but it is not clear whether this is caused by a dysfunction in active mucosal transport or by a bacterial degradation of bile acids that reduces their availability for reabsorption. There is a theoretical increased risk of gallstone formation because the loss of the enterohepatic circulation may reduce the bile acid pool, leading to fat malabsorption.<sup>89</sup> Fat malabsorption has been documented in some studies,<sup>60;90</sup> whereas others have noted normal fat absorption.<sup>6;61;91</sup> No studies have reported an increased incidence of cholelithiasis in pouch patients.<sup>86;92</sup>

One study reported 11% of 83 pouch patients had a low serum vitamin B12 level three years after RP, and 36% showed impaired B12 absorption, as indicated by a low Schilling test result.<sup>60</sup> Other studies have described similar findings.<sup>61;90;91;93;94</sup> It is unclear whether this deficiency is due to; an inadequate intake, a dysfunction of mucosal transport across the terminal ileum or the bacterial binding of vitamin B12 in the pouch, rendering it unavailable for absorption.<sup>60</sup>

Three studies have noted a reduction in carbohydrate and protein absorption following RP. These findings raise the question of whether or not there is a loss of small bowel function following the adaptive changes within the ileal mucosa.<sup>84;90;95</sup>

Results following RP demonstrate that serum folate levels were normal, but a minority of patients were found to have a low haemoglobin level or low serum iron

level.<sup>60;61;84</sup> It is unclear whether these were caused by inadequate intake, impaired absorption, increased requirements or blood loss.<sup>60</sup>

Further research is clearly required with regard to food intake, nutrient absorption and nutritional status of pouch patients.

### **1.1.8 Sexual aspects of pouch surgery**

Following RP, the only visible sign of altered body image, may be the operative scars.

However, as a consequence of the surgery and the resulting physiological changes that the patient undergoes, he or she may feel less attractive and less able to perform sexually. RP has an impact on sexual function, fertility and pregnancy outcome and patients should be counselled on these topics prior to surgery.<sup>79</sup>

Beitz studied 10 patients who underwent RP who described their feelings of “feeling different”, even affected to a significant enough degree for one to describe themselves as a ‘freak’. Patients also reported a reduction in self-respect and confidence. Role and relationship changes highlighted feelings of inadequate spousal relationships and altered intimacy and gender roles. Prior to pouch formation there may already have been body image and sexuality changes resulting from the underlying condition necessitating surgery(e.g. UC) and from a temporary stoma, if it was necessary to form one.

Guerrero described how many clinicians view body image changes as unavoidable and secondary in importance to medical treatment such that the demoralising effects of a patient’s altered body image on sexuality are often not taken into account.

Follow-up studies for pouch patients based on aspects such as body image, clothing, sport, work and sexual appeal, show that about 90% think that their quality of life is better following RP.

In Beitz’s study, over half the respondents reported altered pelvic sensation after pouch surgery. However, the pouch had a marked positive impact on the body image of both male and female respondents, allowing them to feel that they were no longer patients.

In comparison trials, pouch patients feel more satisfied with their body image and sexuality than those with a conventional stoma and report that the ileal reservoir has enhanced their lives.

The pouch offers the patient greater potential for the best possible quality of life by obviating the need for a permanent ileostomy, thus enhancing body image.

Therefore, satisfaction with the pouch procedure has been high. The absence of a permanent ileostomy has eliminated many body image problems, and sexual function following this procedure has not been a major problem.

Studies have shown that a continent procedure improves the quality of sexual life in about 85% of both men and women. It should be an essential component of the post-operative follow-up to ask whether patients undergo sexual difficulties, especially when it is, in the main, younger patients who undergo surgery for FAP or UC. Bond et al. examined the effect of a pouch on personal relationships, particularly sexual relationships, finding that overall almost three-quarters of patients reported an improvement in their sex life.

Post-operative concerns regarding sexuality, especially in women, have not been well catered for. Some women feel more sexual, whereas others have decreased desire.

Vaginal discharge and dyspareunia are well known problems in patients with proctocolectomy and conventional ileostomy but seem less common in pelvic pouch patients. This can be explained by the anatomical changes that take place after proctocolectomy, when the vagina and the uterus fall back and adhere to the sacrum. Following RP, this posterior dislocation is avoided if the pouch replacing the rectum is interposed between sacrum and the internal genitals.<sup>96</sup>

Pelvic nerve damage caused by pelvic dissection to remove the rectum may occur resulting in altered sexual function. The associated changes, including; retrograde



ejaculation, impotence, dyspareunia and vaginal dryness, may be lifelong.<sup>97;98</sup> Pre- and post-operative counselling and support are mandatory to inform patients about these possible complications and to help them cope with the consequences. Male patients must be counselled concerning impotence pre-operatively.

Studies show a 50% reduction in female fertility rates following RP. Since fertility is not affected by subtotal colectomy and end ileostomy formation, it appears that pelvic dissection is the causative factor.

Fertility is therefore, a major consideration of medico-legal importance when advising females who may wish to have children. After counselling, the patient may decide to have a subtotal colectomy and end-ileostomy, as the best strategy for recovery of health and preservation of fertility, leaving a RP for a later date convenient to the patient, when post-partum sphincter integrity may then be known.

Many women receiving a pelvic pouch are young and have concerns about becoming pregnant. They frequently raise the issue concerning their future abilities to conceive and carry a pregnancy to term. Similarly, men may worry about their ability to father a child after pouch surgery.

Pregnancy following pouch surgery is usually possible. Once pregnancy has been achieved, the pelvic location of the pouch may raise concerns in patients and doctors, regarding the effects of the enlarging uterus on pouch function and the best way to deliver the infant. Whereas normal vaginal delivery is possible, a caesarean section avoids the risk to the pelvic floor muscles and nerves and prevents the possibility of needing an episiotomy during delivery.

Scott et al's study<sup>99</sup> to evaluate pregnancy, delivery and functional results for women after RP, reported that 12 out of 330 women who had undergone pouch formation between 1982-1994, were identified as having at least one pregnancy and delivery.

The study concluded that pregnancy and delivery do not affect long term ileal pouch function in most patients. The obstetric outcome in most women following RP is good. This study also found that there is no data to support one particular route of delivery over the other. It is important to bear in mind that evidence also exists suggesting that some patients' fertility may be considerably reduced after surgery for inflammatory bowel disease.<sup>96</sup>



## **1.2 Conditions treated surgically by restorative proctocolectomy**

### **1.2.1. Ulcerative Colitis**

Ulcerative Colitis is a recurrent inflammatory disease of unknown aetiology, affecting the large bowel. It is characterised by clinical features of; rectal bleeding, diarrhoea and abdominal pain. The colonic mucosa becomes inflamed with definable histological features detailed below. These changes invariably affect the rectum and spread, in continuity, to involve a variable amount of the colon. Backwash ileitis occurs in some cases of total colonic involvement but this disappears on treatment. Although the aetiology is unknown, genetic factors are important. Medical treatment can control the disease in most cases but about 30% of patients will progress to surgery.<sup>38</sup>

#### **1.2.1.1 Epidemiology**

UC is a disease predominantly affecting young patients. It affects males and females equally up to the fourth decade of life. Thereafter, the incidence in females declines, whereas it remains much the same in males. The annual incidence per 100,000 population is shown in the table below. Generally the incidence of UC is 10 per 100,000. The prevalence of UC is about 160 per 100,000 population. In the UK, roughly 100,000 patients are affected with UC. There is an increased incidence of UC among Jews and Indian immigrants to the UK.<sup>6</sup>

#### **1.2.1.2 Aetiology**

The aetiology of UC is unknown but its development appears to be influenced by both genetic and environmental factors which probably interact. Kirsner and Spencer<sup>100</sup> described the possibility that UC and Crohn's disease may have a genetic aetiology. A higher prevalence exists amongst "Ashkenazy" compared to "Sephardic" Jews<sup>101</sup>. Between 10-20% of affected individuals have a first-degree relative with IBD.<sup>102;103</sup> The relative risk of a first-degree relative of a proband is ten- to twentyfold.<sup>104</sup>

Any genetic influence is not thought to be Mendelian in nature. Crohn's disease and UC can occur in the same family.<sup>105</sup> Also 10-15% of patients exhibit features of both diseases, labelled "indeterminate colitis" and the change of one disease to the other in a further 10%, may be a feature of genetic heterogeneity. Extra-alimentary manifestations such as ankylosing spondylitis and primary sclerosing cholangitis are more commonly seen in first degree relatives of affected patients; both have HLA associations including HLA-B27 and HLA B8 respectively.<sup>105</sup>

UC is more common in whites than in Afro-Caribbeans or Arabs.<sup>102</sup> However, this does not allow the conclusion to be drawn, that this is a manifestation of genetic susceptibility over environmental influences. IBD has a low incidence in underdeveloped countries but it is apparent that it features more commonly with increasing wealth.

The incidence of UC rises about ten years ahead of Crohn's disease, suggesting that changes in environmental factors do play a role. There is evidence that non-steroidal anti-inflammatory drugs precipitate IBD in humans.<sup>29</sup> They have a direct cellular toxic effect and lead to increased mucosal permeability.

Some patients with UC have previously suffered infective proctocolitis.<sup>106</sup> Smoking is protective in UC<sup>107</sup> but not in Crohn's disease, where it is associated with an increased risk of recurrence.<sup>45</sup> Cessation increases the risk of developing UC and of experiencing an exacerbation, in patients who are in remission. This also appears to be true for pouchitis.<sup>32</sup>

At present it is not known whether UC is a primary immune disorder in a genetically susceptible individual, whether the primary abnormality is outside the immune system but accompanied by an excessive immunological response, or whether it is a combination of both.

### **1.2.1.3 Clinical Presentation**

The large intestine extends proximally from the anal canal, encompassing the entire rectum and colon.. The mucosal columnar glandular epithelium extends into the anal canal to the anal transitional zone, which varies in its longitudinal length, from a few millimetres, to over a centimetre.<sup>108</sup> The anatomical extent of UC varies from involvement of the upper anal canal and rectum alone (proctitis) to the colon more proximally (proctocolitis). The rectum is always involved for all practical purposes, although relative rectal sparing can occur in patients receiving local anti-inflammatory treatment.

At first clinical presentation, the anatomical distribution of disease is variable. Whilst approximately 50% of cases have proctitis alone, in 30% of cases this extends to the left colon (proctosigmoiditis), and in a further 20% disease extends beyond the splenic flexure (extensive colitis). Proximal extension may go no further than the splenic flexure or it may extend all the way to the ileocaecal junction (pancolitis).

The diseased segment of large bowel is affected in a continuous pattern without any intervening segment of normal mucosa.

Patients presenting with proctitis alone describe symptoms of passing blood and mucus per rectum along with increased frequency and urgency of defaecation. These patients do not commonly describe systemic symptoms. They rarely progress to develop a subsequent cancer but their inflamed segment of large bowel may extend proximally with time.<sup>3</sup>

Patients with more extensive disease “proctocolitis,” more commonly suffer with systemic symptoms. Typically, they pass “bloody” diarrhoea associated with frequency and urgency of defaecation. When the latter is severe, there may only be a few seconds warning of imminent defaecation which may result in urge incontinence. Such symptoms exert a very domineering effect on patients’ social and professional lives. Loss of protein and blood in the inflammatory exudates may result in a protein losing enteropathy and

subsequent malnutrition, with loss of lean body mass and anaemia. Growth retardation may affect children with UC. Acutely unwell patients may suffer water and electrolyte depletion resulting in hypovolaemia and also breakdown of the mucosal barrier which may subsequently result in septicaemia.

The pattern of disease is usually one of relapse and remission with exacerbations that may be precipitated by anxiety or stress, but usually there is no recognisable precipitating factor. Some patients experience UC in the form of persisting chronic disease, whereby acute exacerbations, settle only partially on treatment. Patients with more extensive disease are at greater risk of developing colorectal cancer and are more likely to suffer with associated extra-alimentary manifestations. These complications may affect patients with left-sided disease but are more common in those patients whose inflammation extends beyond the splenic flexure.

#### Extra-intestinal manifestations related to disease activity<sup>3</sup>

- erythema nodosum
- pyoderma gangrenosum
- asymmetrical arthropathy
- conjunctivitis
- episcleritis
- uveitis
- thrombo-embolic disease
- amyloidosis
- 

Between one quarter and one third of patients with UC will develop at least one extra-alimentary manifestation during the course of the illness. Arthropathy is the commonest extra-alimentary manifestation affecting patients with UC and can be divided into three broad groups: Activity-related polyarthropathy, ankylosing spondylitis and asymptomatic sacroileitis. Activity-related polyarthropathy is by the most common extra-alimentary manifestation, predominantly affecting the large joints of the limbs, especially the knees. It affects up to 20% of patients and is more likely in those with extensive disease and

disappears when medical treatment induces a remission or after proctocolectomy. It is also recognised in patients affected with pouchitis following RP.

Ankylosing spondylitis, a condition predominantly affecting the sacroiliac joints and one or more vertebrae, is strongly linked to HLA-B27. It affects up to 5% of patients with IBD. The disease is unrelated to the activity of colitis and does not respond to proctocolectomy.

Asymptomatic sacroileitis affects only the sacroiliac joint and is HLA B27 negative. It is more common than ankylosing spondylitis and is unaffected by treatment for colitis.

Associated hepatic and extrahepatic disorders predominantly affect those with extensive colonic disease, recognised in up to 5% of cases. Many patients develop a “fatty liver” which may be precipitated by chronic illness, malnutrition and steroid medication.

Parenchymal liver disease of the chronic active hepatitis type and cirrhosis leading to portal hypertension may occur. Primary sclerosing cholangitis (PSC) affects up to 4% of cases and is more commonly seen in UC than CD. Patients with PSC subsequently undergoing RP, have a higher incidence of pouchitis.<sup>22</sup> Treatment of the hepatic disease of PSC patients with antibiotics, steroids or colectomy proves unsuccessful and ultimately the disease progresses to liver failure. Such patients may be considered for liver transplantation.

Cholangiocarcinoma has a rare association with UC.

In terms of cutaneous manifestations associated with IBD, erythema nodosum is the most common and is more frequently seen in Crohn's disease than UC. Its presence is activity-related. Pyoderma gangrenosum is more commonly seen with UC than Crohn's disease. It usually occurs in the lower limb as a circumscribed area of erythema with a punched-out ulcerated centre. In about half of cases, proctocolectomy seems to be followed by healing over the course of weeks to months.

Of the associated eye conditions, uveitis is rare. It is not related to disease activity.

Episcleritis is activity related and occurs more often in Crohn's disease than UC.

A small proportion of patients(5-10-%) present with acute severe colitis. The patient will be unwell with; severe local symptoms, weight loss, anorexia, water and sodium depletion. Intensive medical treatment may involve corticosteroids, azathioprine or cyclosporine Failure of these drugs to induce remission necessitates urgent surgery.

Acute severe colitis may progress to toxic dilatation and ultimately, perforation. Toxic dilatation refers to hazardous thinning of the bowel wall when the colon is distended beyond diameter of 6cm, seen on plain radiograph. Clinical features may include; distension, localised tenderness and rigidity suggesting localised peritonitis, due to either, irritation of the parietal peritoneum in contact with the inflamed peritoneum or a confined perforation. Patients with a colonic perforation who are on high doses of steroids, may display few of the expected clinical features, although usually the normal features of generalised peritonitis are present. The mortality of a perforated toxic megacolon is over 40% even with optimal treatment. Rectal bleeding rarely necessitates emergency surgery. In this scenario the source of bleeding is usually from ulceration of the rectum and the standard emergency operation of colitis may need to be modified to include removal of the source.

#### **1.2.1.4 Endoscopic findings**

The first noticeable change in the diseased mucosa is mucosal oedema resulting in loss of the vascular pattern. Epithelial oedema leads to the macroscopic change described as fine granularity. Disease progression results in further visible changes including erythema, contact bleeding and occasionally frank ulceration. Where previous acute attacks have been followed by repair, mucosal regeneration nodules or pseudopolyps may be seen. Pseudopolyps represent tags of mucosa that have been partially detached during the active episode and remain as projections after healing of the ulcers.

#### **1.2.1.5 Radiological findings**

The visible radiological changes are helpful in defining the proximal extent of the disease. The severity of the mucosal damage ranges from granularity caused by mucosal oedema to deep ulceration in cases of advanced local disease.

#### **1.2.1.6 Histopathological features**

UC is usually a disease is confined to the mucosa except in fulminant colitis, where inflammatory changes extend through into the muscularis propria. In active disease, diagnostic microscopic features include; mucosal thickening with a heavy infiltration of plasma cells and lymphocytes into the lamina propria. These are accompanied by neutrophils, eosinophils and, mast cells. Mucin within goblet cells is discharged so these are depleted or absent. Severity of active inflammation is best determined histopathologically, by the extent of the neutrophil infiltration. In mild disease, neutrophils are present within the lamina propria. Crypt abscesses are defined by the extrusion of neutrophils into the crypt lumen. The number of crypt abscesses

significantly correlates with the severity of the disease. Ulceration may also be present and may result from crypt abscesses causing mucosal destruction.

Pseudopolyps are oedematous areas of residual epithelium between zones of ulceration. Damage to the crypt basal epithelium leads to loss of crypts. Attempts at regeneration may be mistaken for dysplasia but the presence of more normal cells towards the luminal surface allows these two conditions to be distinguished.

Progression of these acute changes occurs in fulminant colitis. Ulceration may be very extensive, leaving large areas of muscularis propria covered with granulation tissue.

This may be associated with thinning of the musculature and, in toxic dilatation, may show fissure formation within the granulation tissue.

In about 10% of cases, insufficient numbers of diagnostic features are evident to enable distinction between UC and CD and these patients are labelled as suffering from “indeterminate colitis”.<sup>109</sup>

#### **1.2.1.7 Surgery for acute colitis**

The indications for surgery in the management of acute colitis are<sup>38</sup>

- a) Acute severe colitis failing to respond to medical treatment
- b) Megacolon
- c) Perforation
- d) Bleeding



As illustrated in **table 5** below, failure of medical therapy is by far the commonest indication for surgery.<sup>52</sup>

**Table 5** Indications for urgent surgery in ulcerative colitis patients

<u>Indication</u>	<u>No. of patients</u>
Failed medical treatment for severe colitis	71
Toxic dilatation	23
Perforation	9
Bleeding	2
Other	1
<b>Total</b>	<b>106</b>

Factors suggesting that operative intervention is more likely, at the time of admission, include; a frequency of defaecation more than ten times per day with the passage of blood with every defaecation attempt, low serum albumin, low haemoglobin and a fall in lean body mass of more than 10%. Surgery is most frequently required in the first year of onset of the disease.

Megacolon is a definite indication for surgery. If local abdominal signs of peritonism are present, surgery should be performed immediately.

The procedure of choice for acute colitis is colectomy and end- ileostomy with preservation of the rectal stump. It is important to divide the colon at a level allowing exteriorisation of the distal stump, whether this is formed into a mucous fistula or closed. Division at the recto-sigmoid junction is not advocated since it leaves a distal stump which is too short to be exteriorised in the event of breakdown of the suture line and also makes it difficult to identify at a future operation. In acute cases

for bleeding from rectal ulceration, the distal resection margin should be as low as necessary to include the site of the haemorrhage.

Whether the stump is closed or brought out as a mucous fistula depends on clinical judgement during the operation. In a series of 147 consecutive cases there was a mortality of 3% and only 2% experienced leakage from the stump.<sup>110</sup> If the patient's condition is poor and the state of the bowel is deemed too fragile to take sutures or staples, then a mucous fistula is obligatory.

#### **1.2.1.8 Surgery for chronic colitis**

Indications for elective surgery include

- a) Failed medical treatment
- b) Malignant transformation
- c) Retardation of growth in the young

'Failed medical treatment' includes the following clinical settings :

*Chronic colitis:* the patient continues to complain of a combination of local and systemic clinical features, despite adequate medical therapy, including; diarrhoea, rectal bleeding, chronic anaemia associated with general weakness, anergia, amenorrhoea and extra-alimentary manifestations. Prolonged absence from work, disruption of social and family life will increase the likelihood for surgery. Patients who have never experienced a complete remission from medical treatment are included in this group.

*Steroid dependence:* If continual steroid therapy is required to maintain remission and/or relapse occurs on withdrawal surgery is recommended. If alternative

medication such as immunosuppression is unsuccessful, then surgery is indicated unless there are particular reasons against.

*Recurrent acute exacerbations:* Some patients who do respond to medical treatment, develop recurrent relapsing episodes. In such patients, the need for surgery depends upon the frequency of the attacks and their perceived severity. In the acute attack surgery usually takes the form of a colectomy and end- ileostomy with preservation of the rectal stump. Elective surgery for chronic colitis may permit a RP and defunctioning loop ileostomy as the initial procedure.

*Severe symptoms* Urgency of defaecation is usually seen in proctocolitis, due to severe distal inflammation causing rectal irritability and low capacitance. It may be associated with urge incontinence. Failure to control it medically is an indication for surgery.

*Retardation of growth* Extensive UC has an inhibitory effect on growth and on the development of secondary sexual characteristics. Steroids lead to early fusion of epiphyses, resulting in permanent stunting of growth.

*Malignant transformation* The presence of low- or high-grade dysplasia or an established invasive tumour is an indication for surgery

### **1.2.1.9 Surgical options**

There are four surgical options:<sup>38</sup>

- 1) Colectomy with ileostomy and rectal preservation
- 2) Colectomy with ileorectal anastomosis
- 3) Conventional proctocolectomy and permanent ileostomy
- 4) Restorative proctocolectomy and ileal reservoir

Partial colectomy is never advocated, even in cases with a normal right colon where experience has shown a high rate of recurrence in the remaining colon.

The choice of procedure is made after examining the medical criteria and the wishes of the patient. Assessment of the large bowel by radiology, endoscopy and rectal capacitance studies, may assist the clinical assessment to enable a decision as to whether the rectum and anus are suitable for conservation. Colectomy with ileorectal anastomosis should be considered only where the rectum is minimally inflamed and distensible and where there is no evidence of dysplasia anywhere in the large bowel. Most patients do not fulfil these criteria, leaving the options of either conventional or restorative proctocolectomy. The latter is not an option if the anal canal is diseased or if the anal sphincter is inadequate.

The introduction of RP has resulted in a remarkable change in clinical practice with this procedure now being the most commonly employed for these patients.<sup>52</sup>

### **1.2.2 Familial Adenomatous Polyposis FAP**

FAP is a rare autosomal dominantly inherited condition, the gene for which has been found on the long arm of chromosome 5.<sup>111</sup> It affects around 1 in 10 000 live born and is usually characterised by<sup>38</sup>

- 1) Hundreds of colorectal adenomatous polyps at a young age (second or third decade of life)
- 2) Duodenal adenomatous polyps
- 3) Multiple extra-intestinal manifestations
- 4) Mutation in the tumour suppressor adenomatous polyposis coli (APC) gene on chromosome 5q
- 5) Autosomal dominant inheritance pattern (offspring of affected individuals have a 1 in 2 chance of inheriting FAP)

The location of the mutation in the APC gene can influence the FAP phenotype.<sup>14</sup> The resulting phenotypes may exist at different ends of the spectrum between dense colorectal polyposis (severe FAP) and 'attenuated' polyposis (less severe FAP)<sup>15</sup> However, in separate individuals, an identical mutation may result in different phenotypic expression, suggesting that other modifying genes and the environment play a role in disease severity and expression.<sup>112</sup> Importantly, inadequate colonoscopy can lead to a false diagnosis of attenuation, an error that can be avoided by the use of dye-spray.<sup>34</sup>

Nevertheless, there is molecular support for the existence of the attenuated variant, based on the finding that the gene product in attenuated FAP interferes only weakly with APC gene expression when compared with the gene product associated with a mutation in the area associated with severe FAP (codon 1309)<sup>98</sup>. The presence of

different extra-colonic manifestations of FAP, listed below, also partially correlate with the mutation site.<sup>38</sup>

#### Ectodermal origin

- epidermoid cysts
- pilomatrixoma
- Central nervous system tumours
- Congenital hypertrophy of the retinal pigment epithelium

#### Mesodermal origin

- Connective tissue; desmoid tumours, excessive adhesions
- Bone; osteoma, exostosis, sclerosis
- Dental; dentigerous cyst, odontoma, supernumary teeth, unerupted teeth

#### Endodermal origin

- Adenomas/carcinomas of duodenum, stomach, small intestine, biliary tract, thyroid, adrenal cortex
- Fundic gland polyps
- Hepatoblastoma

Currently, all patients with FAP require prophylactic colectomy as the only sensible treatment option. Without treatment, the malignant transformation from adenomas to cancers, is inevitable within about 20 years. Consequently, colectomy is usually performed in the second decade of life and there are three surgical options:

- 1) Colectomy and ileorectal anastomosis(IRA)
- 2) Restorative proctocolectomy with an ileal pouch-anal anastomosis
- 3) Total proctocolectomy and terminal ileostomy

The first option carries the advantages of being a one stage procedure with low morbidity and mortality and usually results in excellent faecal continence. The negative aspects of this procedure include; a cumulative rectal cancer risk of up to 30% by 60 years of age and subsequent proctectomy rate of up to 30%.

RP is most commonly a two stage procedure with a higher morbidity rate than above with subsequent risk of pouch removal. It is associated with worse faecal continence and lower patient satisfaction than patients undergoing the same operation for UC<sup>54</sup> because FAP patients have often never had symptomatic disease. RP is a more complex surgical procedure requiring a greater degree of surgical expertise. Advantageously, it carries a very low rectal cancer risk- especially if combined with mucosectomy and hand-sewn anastomosis. Surveillance follow-up is required after all procedures. After either IRA or RP, perianal, digital and endoscopic examination is mandatory at intervals of up to 12 months depending on findings. In recent years, medical treatment of FAP patients with the non-steroidal anti-inflammatory drug sulindac has been used to control rectal stump adenomas<sup>54</sup> and pouch adenomas<sup>104</sup>. The COX-2 inhibitor celecoxib has shown a moderate reduction in large bowel polyps in treated patients,<sup>104;113</sup> but must be caused with caution in view of the occurrence of cancer despite surveillance in this setting. Following colectomy, the major causes of mortality and morbidity are duodenal cancers, desmoid tumours and in those who have an ileorectal anastomosis, rectal stump cancers.

### **1.2.3. Slow Transit Constipation**

Idiopathic Slow Transit Constipation (STC) is a clinical syndrome characterised by intractable constipation that is not readily responsive to laxatives, diets or change of lifestyle. It is characterised by delayed colonic transit without underlying systemic disorder or pelvic floor dysfunction<sup>5,114</sup>. In addition to the constipation, patients with STC present with other gastrointestinal symptoms such as bloating, abdominal pain, cramps, nausea and vomiting<sup>115</sup>. It is difficult to distinguish these patients from patients with irritable bowel syndrome (IBS) according to Rome II criteria<sup>116</sup>, however patients with STC have a slow colonic transit.

Patients with STC are a small fraction of the total population complaining of constipation<sup>33</sup>. Thus, in tertiary referral centres about one fifth of 91 patients<sup>117</sup> and about two-fifths of 70 had STC<sup>118</sup>. Despite the low number of these patients, the intractability of their symptoms cause psychological and social stress and greatly impair their quality of life. Furthermore, they consume a disproportionate amount of medical resource. It appears that some of these patients are also suffering from a generalised gastrointestinal motility disorder<sup>115</sup>.

Patients with STC are a heterogeneous group and understanding the pathophysiology of this disorder is essential for proper treatment strategy.



### **1.2.3.1 Diagnosis**

A full history, especially concerning pelvic surgery and childbirth, clinical examination and several investigations should be performed in order to reach the diagnosis. Blood tests should be taken to exclude metabolic, endocrinological or systemic diseases. Defaecating proctography, rectal manometry and anal sphincter electromyography are performed to exclude rectocoele, enterocoele, intussusception, paradoxical puborectalis contraction and other pelvic floor disorders<sup>119</sup>. Colonic transit time is essential for the diagnosis. Several techniques are used to estimate the colonic transit time; radiological, isotopic or biomagnetic<sup>12;13;24;34;101;104;105;114;120-122;122-126</sup>. The radio-opaque markers method with single plain abdominal X-ray is simple, safe and reproducible<sup>126;127</sup>. The methods reported by Metcalf<sup>121</sup> and Abrahamsson et al.<sup>123</sup> are the most popular. Radio-opaque marker methods are not considered to be sufficiently discriminatory in showing different segments of transit delay.<sup>114</sup> Colonoscopy or barium enema is necessary to exclude megacolon and megarectum as well as malignancy.

### **1.2.3.2 Pathophysiology**

Dysmotility of the colon and in some patients a generalised gastrointestinal motility disorder seem to be the cause of STC.<sup>33;128</sup> The cause of this dysmotility is debatable.

Selective small fibre neuropathies have been found in 12 patients with STC.<sup>129</sup> It has been proposed that STC arises as a consequence of pelvic autonomic dysfunction<sup>130</sup>. This dysfunction would arise after pelvic surgery such as hysterectomy, tubovarian and even appendicectomy, as well as after childbirth.<sup>130</sup> In idiopathic cases, the cause has been suggested to be neuronal degeneration in the pelvic autonomic nervous system.<sup>130</sup> Although this hypothesis is based on circumstantial evidence, it appears to be applicable to a considerable number of patients with idiopathic STC. Thus, of 31 patients with idiopathic STC, 14 have been reported to develop severe constipation following a hysterectomy.<sup>131</sup> In another study, 12 of 48 patients with idiopathic STC developed symptoms following pelvic surgery or childbirth.<sup>132</sup>

Defaecation is voluntary and can be suppressed or delayed. Social convenience or other psychological factors may affect the frequency of defaecation. It has been reported that personality type might influence stool weight and frequency.<sup>133;134</sup> Co-existing psychiatric problems such as depression are less common in patients with STC than in constipated patients with normal colonic transit.<sup>79</sup> Although constipated patients have more psychological stress than healthy subjects, idiopathic STC patients have less dimensions of hypochondriasis and disease affirmation.<sup>135</sup> Psychosocial factors may contribute to the aggravation of the disease, but can not be considered as a major factor for the development of idiopathic STC.

### 1.2.3.3 Management

As these patients are a heterogeneous group, treatment should be individualised.

The treatment of patients with idiopathic STC has been the subject of recent comprehensive reviews.<sup>41;114;128;135;136</sup>

Clinical experience shows that the benefits of continuous intake of laxatives (which are effective in the short term) tends to decline with time.<sup>135</sup> El-Salhy et al. describe alternating between two drugs in order to reduce side effects and reduce and avoid dependence.<sup>118</sup> Dietary fibre supplements and osmotic laxatives that consist of unabsorbed sugars, are generally ineffective<sup>137</sup> and may aggravate nausea, abdominal pain and bloating in these patients.

The drugs available and used in patients with idiopathic STC are stimulant laxatives such as bisacodyl, non-gas producing osmotic laxatives including polyethylene glycol, saline laxatives such as milk of magnesia, and enemas. Colchicine and misoprostol can also be used.

Erythromycin is a motilin receptor agonist.<sup>138</sup> Some patients with idiopathic STC exhibit reduced basal and post-prandial plasma levels of motilin. Erythromycin in low doses has been found to stimulate distal colonic motility in these patients.<sup>139</sup>

Surgical treatment has been based on the belief that idiopathic STC is caused by colonic stasis. Thus, total colectomy, subtotal colectomy with ileorectal, caecorectal or ileosigmoid anastomosis as well as segmental resection (hemicolectomy) have been used.<sup>29;140-145 146-148</sup> The most common operation used for idiopathic STC is total colectomy with ileorectal anastomosis.<sup>129</sup> Subtotal colectomy with caecorectal anastomosis has been proposed to be superior to the other operations, as it spares the distal ileum, ileocaecal junction and caecum. This reconstruction preserves important functions such as absorption of water, bile,

vitamin B12 and electrolytes.<sup>149</sup> Subtotal colectomy with antiperistaltic caecorectal anastomosis without wide mobilisation of the right colon or torsion to the vascular pedicle has been applied with good short-term post-operative results.  
144;150

The incidence of post-operative complications in idiopathic STC are variable.<sup>140</sup> However, long-term (5-12 years follow-up of patients with idiopathic STC, who had undergone colectomy with ileorectal anastomosis, has reported a high complication rate.<sup>151</sup> Thus, 71% of the patients had at least one episode of small intestinal obstruction and 42% of these episodes resulted in laparotomy.<sup>151</sup> Comparison of the outcome in idiopathic STC and that in patients with UC and colonic carcinoma, who had undergone colectomy, has shown that post-operative complications are significantly higher in patients with idiopathic STC.<sup>151</sup> Patients with idiopathic STC after hysterectomy or childbirth with normal proximal gastrointestinal function seem to be suitable for surgical management.<sup>152</sup> Biofeedback is advocated as a method for treating patients with intractable constipation.<sup>153-156</sup> The mechanism of this therapy effect is unclear and contingent upon who performs it, how often and how it is performed.<sup>157</sup> It appears that this treatment serves a more global psychological counselling function.<sup>157</sup> Whereas the success of biofeedback treatment has been reported to be between 30 and 40%.  
158-160 Randomised controlled trials of STC patients with this mode of therapy are few.<sup>161</sup> Until a larger group of patients with idiopathic STC are examined in randomised controlled studies with a long follow-up, the efficacy of this treatment remains uncertain.

## **CHAPTER TWO**

# **“CUFF” SURVEILLANCE AND THE USE OF P53 AS A PRE- DYSPLASTIC MARKER FOLLOWING RESTORATIVE PROCTOCOLECTOMY FOR ULCERATIVE COLITIS**

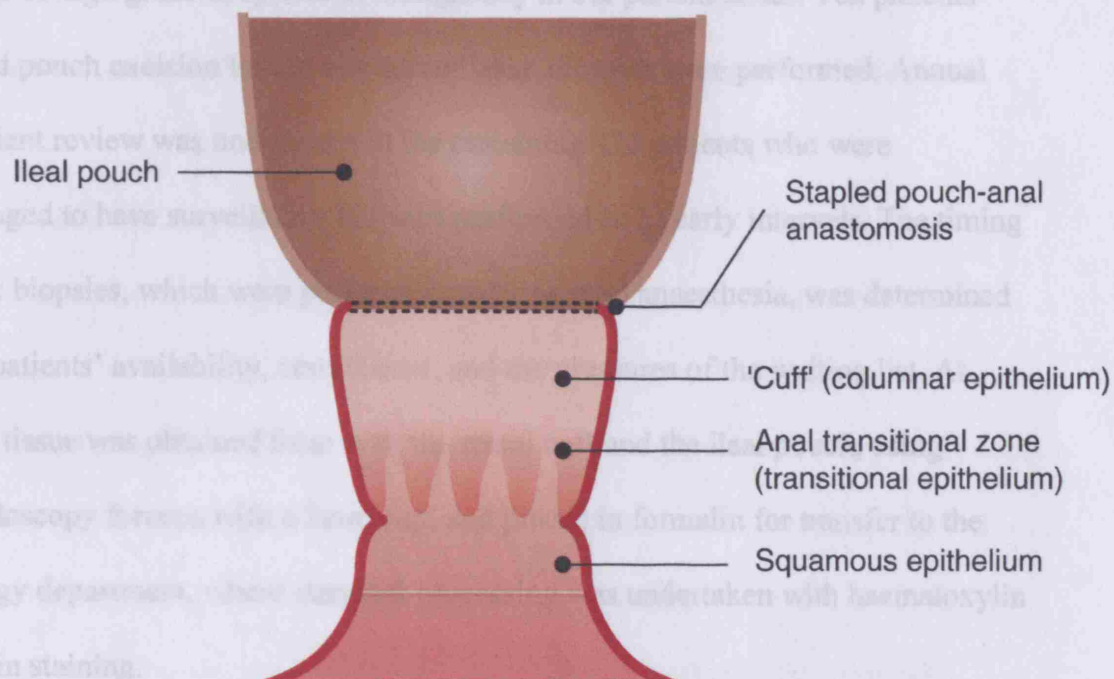
## 2.1 Introduction

Restorative proctocolectomy is the operation of choice for most patients with UC requiring surgery.<sup>162;163</sup> Patients with chronic UC have an increased risk of developing colorectal carcinoma<sup>164-166</sup> when compared with control subjects. The presence of colonic dysplasia has been shown to be a risk factor which warrants careful follow up.<sup>167;168</sup>

This risk also applies to patients with UC who have had surgery, if rectal mucosa is retained. After colectomy and ileo-rectal anastomosis (IRA) it has been calculated that there is a 6% risk of developing a rectal cancer during the subsequent 10-20 years,<sup>169-174</sup> rising to 15-20% after 30 years.<sup>169;175</sup> In the initial description of the procedure in 1978<sup>3</sup>, resection of all the rectal mucosa was used to eliminate the recognised risk of malignant change in colitic mucosa. The availability of stapling instruments has simplified the operation and improved the functional results by retaining the anal-transition zone.<sup>175</sup> In stapled RP a “cuff” of columnar rectal epithelium is retained from the top of the anal transitional zone to the level of the pouch-anal anastomosis, which has the potential risk of malignant change. Consequently, regular surveillance biopsies of the rectal cuff are taken, but it is unknown how frequently these biopsies should be performed or how soon after surgery they should commence. In 1988 we adopted the RP technique and instituted a follow up surveillance program of cuff biopsies. The results to date are reported.

**Figure 6** Anatomy of the mucosal “cuff” following restorative proctocolectomy

## The Mucosal 'Cuff' and Anal Transitional Zone



### **2.3 Patients and methods**

135 patients underwent RP for ulcerative colitis between 1988-98. Stapled anastomoses without mucosectomy were performed for all cases because there were no cases of high grade dysplasia or malignancy in our patient series. Ten patients required pouch excision before any surveillance biopsies were performed. Annual out-patient review was undertaken in the remaining 125 patients who were encouraged to have surveillance biopsies performed at 2 yearly intervals. The timing of these biopsies, which were performed under general anaesthesia, was determined by the patients' availability, compliance, and the pressures of the waiting list. At biopsy, tissue was obtained from both the rectal cuff and the ileal pouch, using sigmoidoscopy forceps with a 2mm cup, and placed in formalin for transfer to the pathology department, where standard processing was undertaken with haematoxylin and eosin staining.

All specimens were reviewed by a single pathologist with a specialist interest in inflammatory bowel disease, in three sessions, in order to preserve consistency of reporting. Slides were examined to confirm the presence of columnar rectal epithelium and then subjectively graded for the degree of acute and chronic inflammation in the rectal cuff.



## **2.4 Definitions of “cuff” inflammation and dysplasia**

Acute inflammation was considered to be present when polymorphs were identified in the epithelial surface and lamina propria.

Mild acute inflammation was defined as the presence of occasional polymorphs in a crypt.

Moderate acute inflammation was defined as the presence of overt cryptitis, and

Severe acute inflammation was defined as the presence of crypt abscesses or frank ulceration.

Mild chronic inflammation was defined as the presence of a scattering of plasma cells.

Moderate chronic inflammation was defined as the presence of denser aggregations of plasma cells throughout the entire thickness of the mucosa and

Severe chronic inflammation was defined as the intense packing of plasma cells throughout the mucosa.

All specimens were examined for the presence of dysplasia or carcinoma according to standardised criteria<sup>176</sup> and classified as : no dysplasia, reactive dysplasia, low grade dysplasia (LGD), high grade dysplasia (HGD) or carcinoma.

Reactive dysplasia described histological changes caused by an inflammatory reaction or by regenerative activity that was not frankly dysplastic.

LGD described cellular atypia including nuclear polymorphism, hyperchromatism, nuclear stratification and inverted goblet cells with or without architectural distortion which is not attributable to either inflammation or regeneration.

HGD described severe cellular atypia usually associated with architectural distortion.

## 2.5 Results: Incidence of dysplasia and carcinoma in cuff biopsies following restorative proctocolectomy for ulcerative colitis

Low grade dysplasia was present in three of the original 135 procto-colectomy specimens. None showed any evidence of high grade dysplasia or carcinoma. Ten of these 135 patients required pouch removal within 2 years of surgery and did not have surveillance biopsies.

173 cuff biopsies were attempted in 110 of the remaining 125 patients (15 failed to attend for surveillance). Only 112 of these 173 biopsies contained columnar epithelium from the rectal cuff; the remainder showed ileum or anal skin. The median elapsed time since the diagnosis of ulcerative colitis was 8.8 years (range 2-32) and the median elapsed time since pouch construction was 56 months (range 12-145).

None of the biopsies from the cuff showed dysplasia or carcinoma. Reactive atypia however, was found in 30 of the 112 cuff biopsies (27%).

The majority of cuff biopsies (94%) showed evidence of *chronic* inflammation (**Table 6**), and in over one third (35%) of the biopsies this was severe. Severe *acute* inflammation was found less frequently but was present in one quarter of specimens (25%).

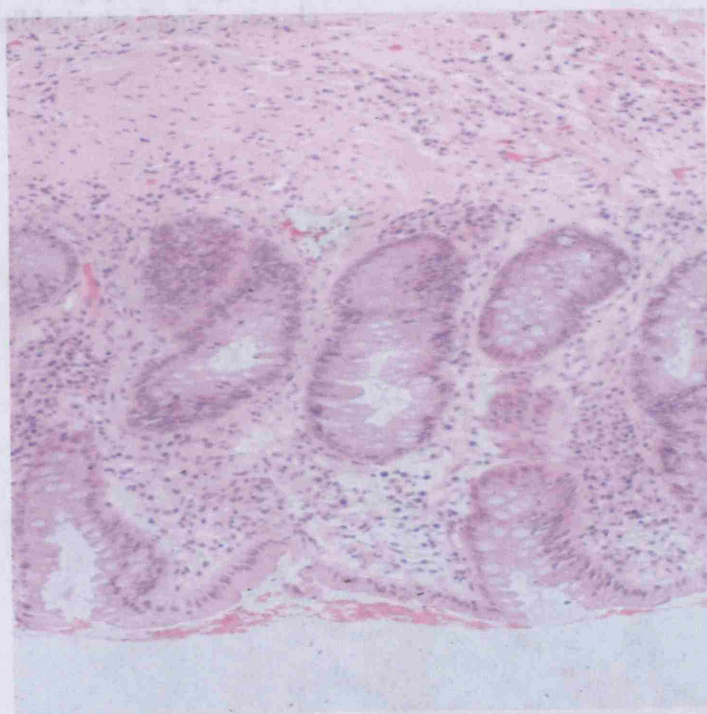
**Table 6** Incidence and severity of acute and chronic inflammation in “cuff” surveillance biopsies

	<u>Active inflammation</u>				Total
	None	Mild	Moderate	Severe	
<u>Chronic inflammation</u>					
None	7(6)	0(0)	0(0)	0(0)	7(6)
Mild	8(7)	21(19)	1(1)	2(2)	32(29)
Moderate	0(0)	17(15)	11(10)	6(5)	34(30)
Severe	0(0)	9(8)	10(9)	20(18)	39(35)
Total	15(13)	47(42)	22(20)	28(25)	

**Figure 7**

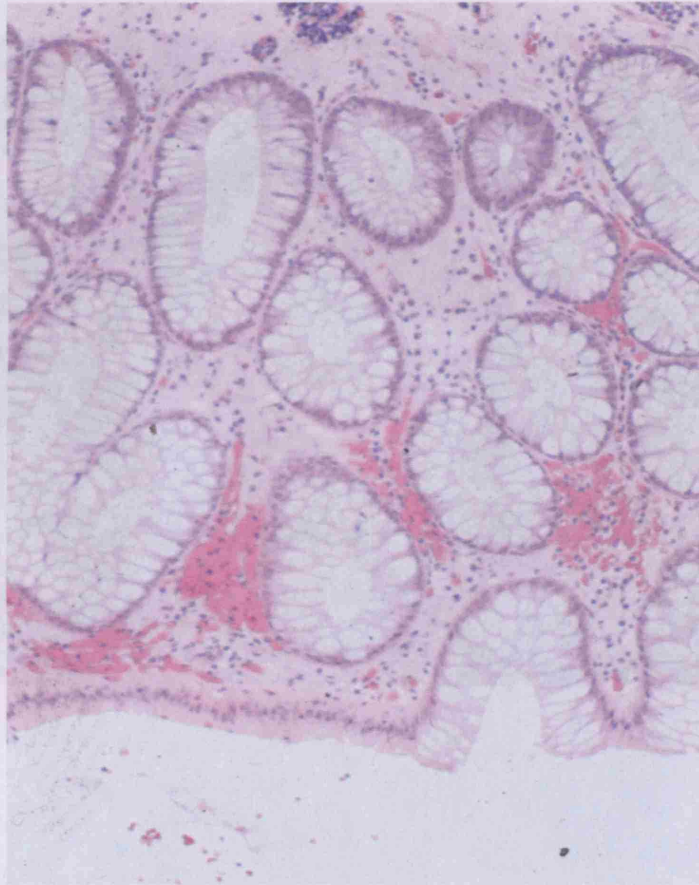
Normal cuff biopsy

Cuff biopsy showing mild active inflammation with polymorphs

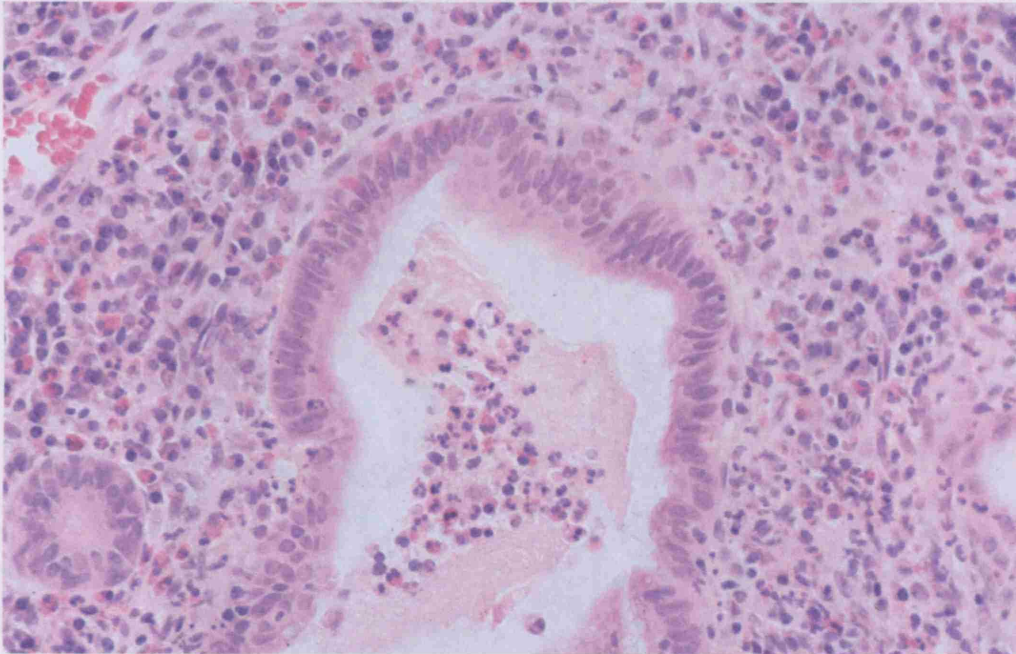


**Figure 8**

Cuff biopsy showing mild active inflammation with polymorphs visible in the lamina propria

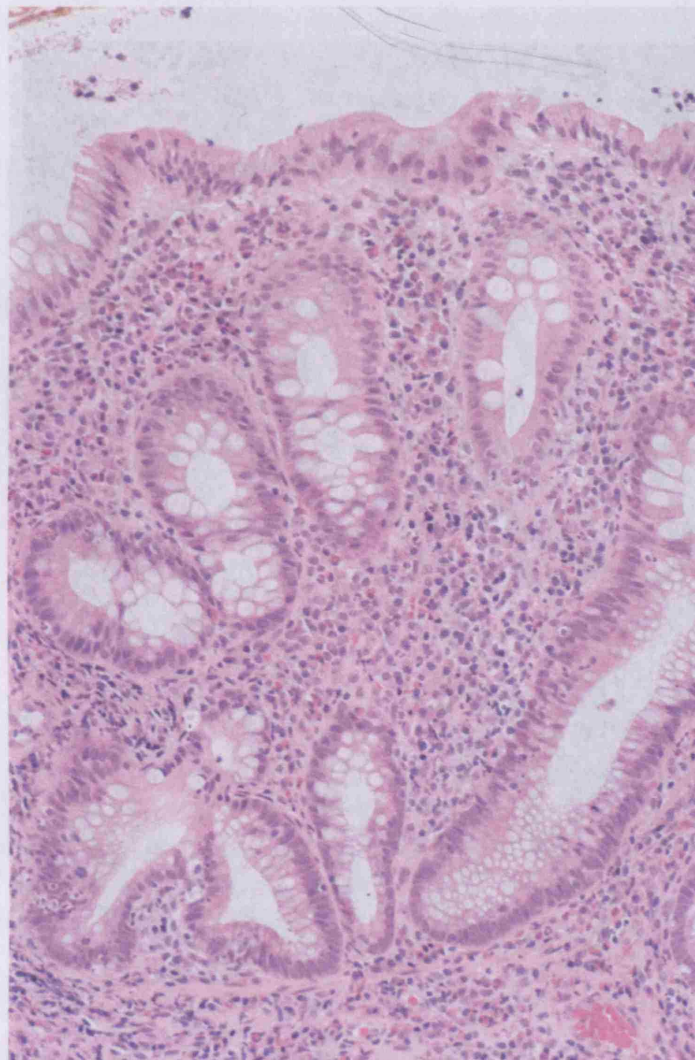


**Figure 9** Cuff biopsy showing severe active inflammation and moderate/severe chronic inflammation

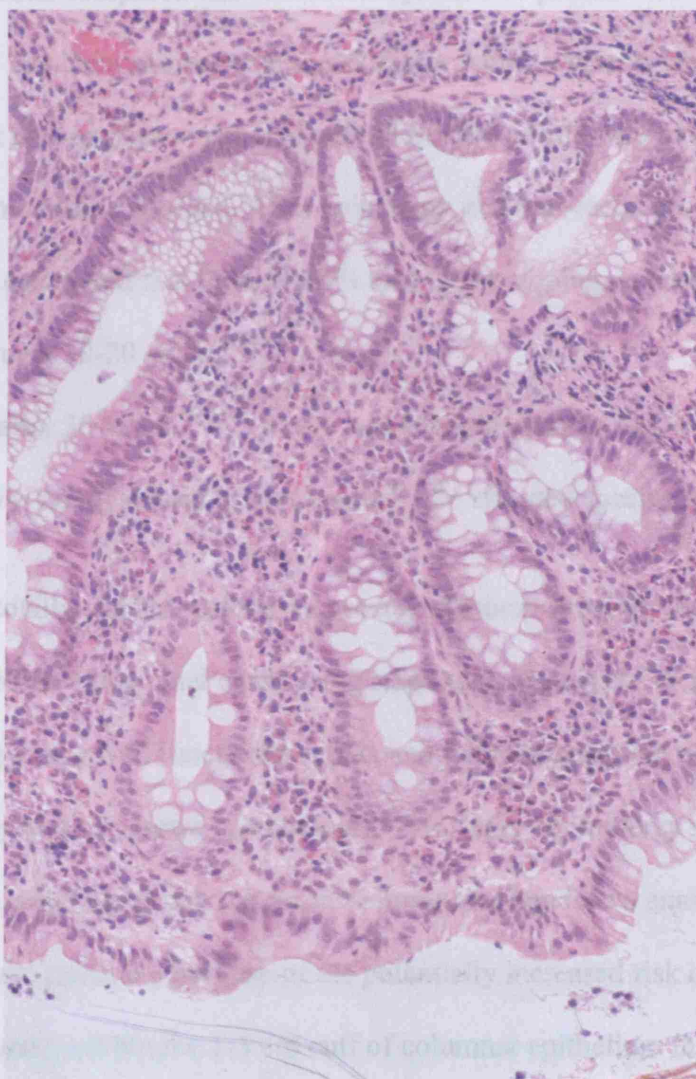




**Figure 10** Cuff biopsy showing severe chronic inflammation with crypt distortion and regenerative activity



**Figure 11** Cuff biopsy showing severe chronic inflammation illustrated by early cryptitis and moderate active inflammation



## 2.6 Discussion

Patients with chronic UC have an increased risk of developing colorectal carcinoma<sup>164-166</sup> when compared with control subjects. The presence of colonic dysplasia has been shown to be a risk factor which warrants careful follow up.<sup>167;168</sup> This risk also applies to patients with ulcerative colitis who have had surgery, if rectal mucosa is retained. After colectomy and ileo-rectal anastomosis (IRA) it has been calculated that there is a 6% risk of developing a rectal cancer during the subsequent 10-20 years,<sup>169-174</sup> rising to 15-20% after 30 years.<sup>169;175</sup> It was noted that the cancer risk was reduced if there was no dysplasia or carcinoma in the resected specimen.

The operation of totally stapled restorative proctocolectomy involves the anastomosis of an ileal pouch to the upper anal canal using stapling instruments. Its proponents highlight its safety, improved functional results and reduced operating time compared with mucosectomy and handsewn anastomosis.<sup>53</sup> However controversy still exists between surgeons, whether to perform mucosectomy and handsewn anastomosis or a stapled anastomosis, primarily because of the potentially increased risk of development of cancer within the 1-3 cm cuff of columnar epithelium that is retained. The hypothetical risk for these pouch patients, extrapolated from the available data for patients with UC who have had a IRA, has been calculated to be 1% at 30 years.<sup>36</sup> This would double to 2% if an additional 2 cm of rectal mucosa was inadvertently retained above the anal canal. Despite these low theoretical risk rates for malignant change in the rectal cuff, it has been considered necessary to follow patients using surveillance biopsies.



In the present study there was no evidence of either dysplasia or carcinoma in the rectal cuff at up to 12 years post pouch formation and up to 32 years post diagnosis of UC. These data from patients are similar to those predicted by the theoretical analysis above. To date there have been eight published series from six centres, which report the risk of dysplasia and carcinoma in cuff biopsies which are shown in **table 7**.

**Table 7** Summary of studies analysing the incidence of dysplasia in cuff surveillance biopsies

<u>Reference</u>	<u>No. of patients</u>	<u>Mean follow-up (months)</u>	<u>Persistent dysplasia</u>	<u>Carcinoma</u>
O’Riordan <i>et al.</i> <sup>177</sup>	210	77	2	0
Remzi <i>et al.</i> <sup>178</sup>	178	130	2	0
Schmitt <i>et al.</i> <sup>{606}</sup>	38	8.4	0	0
Emblem <i>et al.</i> <sup>179</sup>	19	36	0	0
Slors <i>et al.</i> <sup>180</sup>	6	18	0	0
T-Fawcett <i>et al.</i> <sup>36</sup>	109	30	0	0
Haray <i>et al.</i> <sup>181</sup>	109	31	0	0
<b>Present series</b>	<b>135</b>	<b>56</b>	<b>0</b>	<b>0</b>

Although the follow up is short in most of these studies, taken together, only four patients had persistent evidence of dysplasia in the cuff. In two cases this was low-grade dysplasia and both patients subsequently underwent mucosectomy.<sup>182</sup> Further, one of these patients had high grade dysplasia and the other, low-grade dysplasia, in the original colectomy specimen. O’ Riordain *et al.*<sup>177</sup> reported two patients with persistent evidence of high grade dysplasia in the cuff. Both patients had either high grade dysplasia or carcinoma in the original colectomy specimen.

One of these studies estimated the risk of developing cuff dysplasia at 10% if there was dysplasia in the colectomy specimen, and 25% if there was carcinoma in the

original specimen<sup>177</sup>. None of the patients in the present study had either high grade dysplasia or carcinoma in the original resection specimens which may explain why we have not found any dysplasia during follow-up.

If cuff biopsies revealed evidence of high grade dysplasia we would recommend multiple biopsies at intervals of 3-6 months and excision of the anal canal if these features persist. In the case of low grade dysplasia in the cuff, we would recommend six-monthly surveillance biopsies. If these features persisted on three successive biopsies, we would then recommend mucosectomy and perineal pouch advancement.

In the present study rigid instruments and general anaesthesia were used to obtain biopsies. Histological evidence of the “cuff” was found in only 65% of cases showing how difficult it can be to reliably identify the cuff, especially when it is very short. The use of fibre-optic instruments for this purpose may be more accurate in obtaining cuff mucosa and more comfortable for the patient. Although no dysplasia was found in the rectal cuff in the present series, most patients had chronic inflammation. This “cuffitis” may lead to severe symptoms including pain, urgency and bleeding. Indeed the latter may rarely cause profound anaemia necessitating the technically difficult operation of mucosectomy and pouch advancement<sup>183</sup>. It is of note that in the present study, although forty seven(42%) patients had evidence of severe inflammation (acute or chronic), only sixteen(14%) had symptoms requiring medical therapy and two(2%) required surgical intervention. In previous reports, symptomatic cuffitis was found in 2-15% of patients,<sup>179-181</sup> only one of these required surgery. These results are clinically important for the management of patients who have had a totally stapled restorative proctocolectomy for ulcerative colitis. Those patients with no evidence of dysplasia or carcinoma in the resected large bowel probably do not need surveillance biopsies of the rectal cuff in the first 5-

10 years after surgery. This will save both, resources and reduce the discomfort/inconvenience for patients . These data should not be extrapolated to patients who have either high-grade dysplasia or carcinoma in the resected large bowel since there is evidence that these patients have a high risk of developing dysplasia in the cuff . Regular surveillance biopsies should continue to be offered to these patients until further information is available. In the event that it is known that dysplasia or carcinoma is present in the colon prior to surgery then a RP should probably be avoided and a hand-sewn anastomosis performed after removal of the entire anal canal mucosa. In the event that a patient who has had a RP develops dysplasia in the cuff it is unclear how this should be managed . In particular it is unknown whether low grade dysplasia invariably progresses to high grade and then to carcinoma. In one study although eight of 254 patients had low grade dysplasia,<sup>177</sup> only two of these patients had evidence of dysplasia in subsequent in biopsies. Similarly 15 of 22 patients with LGD in another study<sup>167</sup> showed no evidence of dysplasia on repeat biopsy. Low grade dysplasia should therefore be observed by repeat biopsy. In the event that HGD is found in the cuff, the risk of conversion to a carcinoma is unknown but given the present state of knowledge consideration should be given to performing a mucosectomy and pouch advancement.

## **2.7 Pre-dysplastic markers for carcinoma development on a background of ulcerative colitis**

Epithelial dysplasia in chronic UC is the most important marker of an increased risk of malignancy<sup>166;184-188</sup> and our previous work showed

no evidence of either dysplasia or carcinoma in 173 cuff biopsies taken from 110 patients who underwent RP for UC, up to twelve years post-operatively<sup>189</sup>.

However, the failure of surveillance programs to consistently reduce cancer-related mortality, likely results from a combination of poor patient adherence and the inadequacy of using only dysplasia as a marker of cancer risk.<sup>190-193</sup>

<sup>194</sup> <sup>195</sup> Recent studies suggest that p53 deletions or mutations may occur at an earlier phase of UC-associated neoplasia when compared with sporadic

carcinogenesis.<sup>185;186;196-198</sup> Burner et al's work on p53<sup>185</sup>

reported that significant genetic abnormalities may exist in biopsy specimens

that cannot yet be histologically identified as dysplastic. This group, along with

Brentnall et al.<sup>199</sup> endorsed p53 as a pre-dysplastic marker in UC. In one

report of chronic UC patients, p53 mutations were noticed in up to 30% of biopsy

specimens from non-dysplastic epithelium<sup>199</sup> and Lashner et al<sup>200</sup> reported that

p53 mutations occurred early in the dysplasia-carcinoma sequence, before dysplasia

was detected. This finding is supported by a series of mapping studies.<sup>199;201</sup>

<sup>185</sup>.

P53 is a tumour-suppressor gene located on chromosome 17p13.1<sup>196;202</sup>. When

functional, the p53 protein arrests the entry of cells sustaining DNA damage into the

'S' phase of the cell cycle, thereby providing an opportunity for DNA repair to

proceed through normal repair mechanisms, whereas it triggers apoptosis in cells in

which DNA damage is irreversible. Loss of these protective mechanisms might

thereby predispose abnormal epithelia to malignancy, especially under conditions of persistent epithelial injury and turnover, such as those prevailing in UC.<sup>203</sup> Mutations in the p53 gene result in a stable protein product that has a long half-life and is detectable by immunohistochemical methods.<sup>10;184</sup> In 1988 we adopted the stapled-RP technique and instituted a follow up surveillance program of cuff biopsies. We have studied p53 expression, dysplasia and carcinoma in cuff surveillance biopsies obtained prospectively and the results to date are reported.

## **2.8 Patients and method of p53 staining of cuff surveillance biopsies following RP for UC**

The same patient group of 135 patients who underwent RP for UC between 1988-98, as detailed above, was studied. P53 immunohistochemistry was performed on histological specimens cut from blocks containing their “cuff” biopsies.

After the presence of cuff mucosa was confirmed by the histopathologist, immunohistochemistry for p53 expression was performed on each patient’s most recent cuff biopsy.

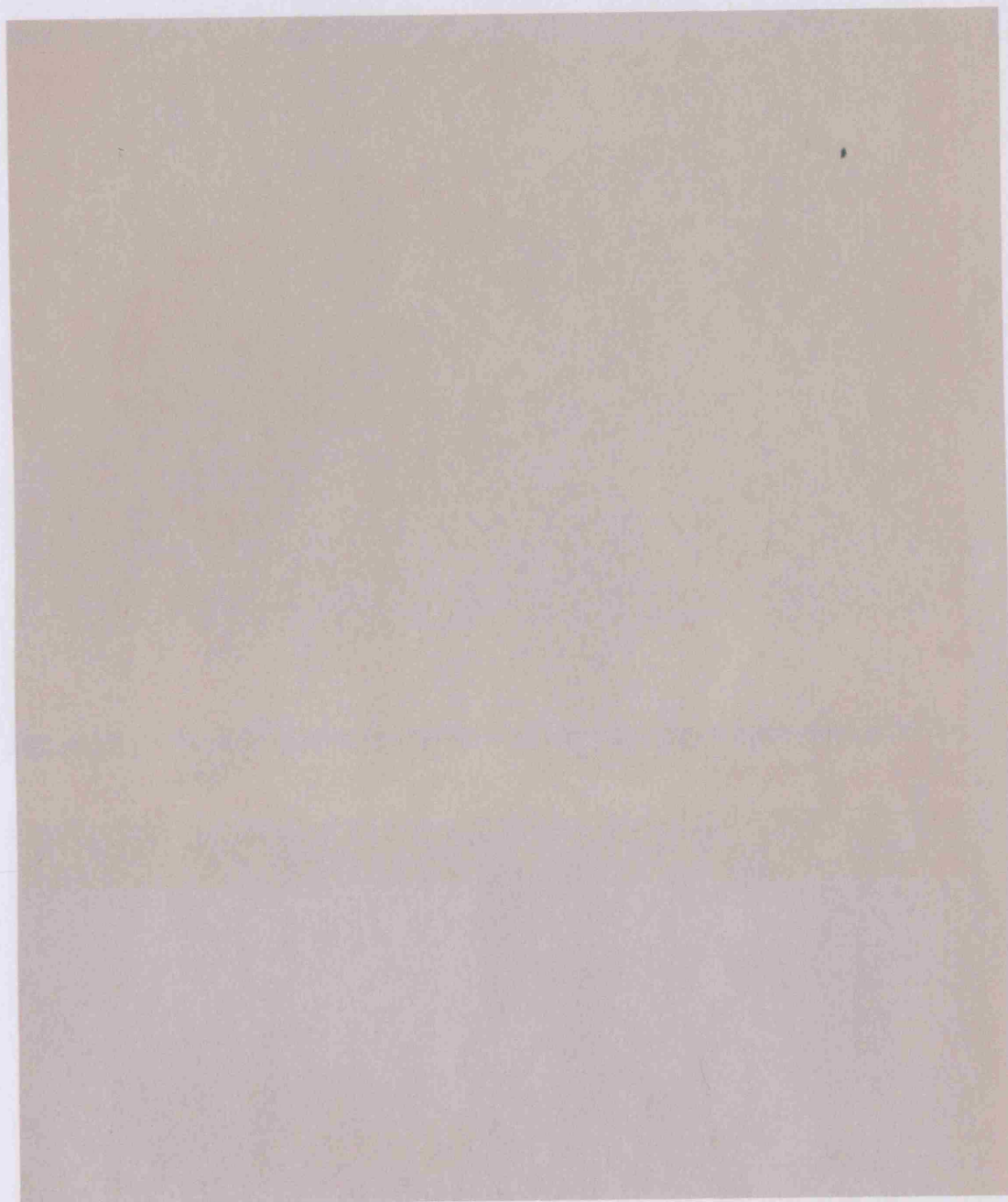
### Immunohistochemistry

Standard immunohistochemistry was performed by the ABC method using a Vectastain **ABC** kit (Vector Laboratories) based on the **A**vidin and **B**iotinylated horse-radish peroxidase macromolecular **C**omplex method (ABC). Fresh 4µm-thick sections were cut from the paraffin blocks of the cuff biopsy specimens. Sections were deparaffinised in xylene and rehydrated through graded alcohols. After immersion in distilled water, specimens were transferred to an acetate buffer (pH 6.0) solution for antigen retrieval and microwaved for 25 minutes. After cooling,

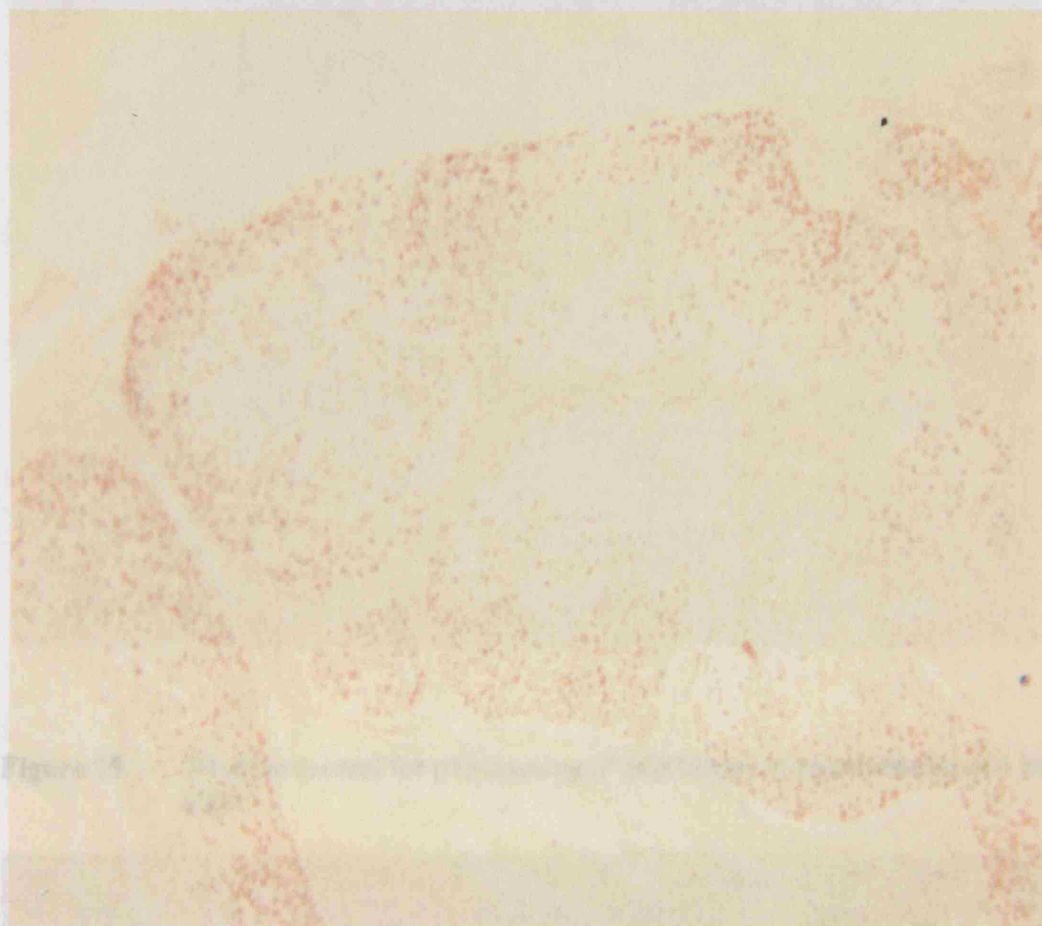
endogenous peroxidase activity was blocked by incubating for twenty minutes with 1% hydrogen peroxide in methanol. After pre-incubating for thirty minutes in normal horse serum, sections were incubated for one hour after application of the mouse monoclonal anti-p53 antibody DO7 (DAKO) specific for both mutant and wild type p53, at a dilution of 1:1000. After washing, specimens were incubated with the biotinylated secondary antibody, anti-mouse immunoglobulin G . Bound antibody was detected using the Vectastain ABC kit in accordance with the manufacturer's instructions with diaminobenzidine as the chromogen. Sections were then counter-stained with haematoxylin, dehydrated through graded alcohols and mounted in DPX (BDH) and reviewed. Biopsy specimens were considered positive for p53 overexpression if more than 10% of the epithelial cells in a biopsy specimen exhibited dark brown intranuclear staining.

For a negative control the primary antibody was omitted and for the positive control specimens were taken from a known p53 positive breast carcinoma. Other controls consisted of sections cut from stapled haemorrhoidectomy donuts and biopsies of ; inflamed UC, normal ileal pouch and normal rectum.

**Figure 12** Negative control for p53 staining of cuff biopsy



**Figure 13** Positive control for p53 staining of cuff biopsy at concentration of 1 in 500





**Figure 14** Positive control for p53 staining of cuff biopsy at concentration of 1 in 1000



**Figure 15** Positive control for p53 staining of cuff biopsy at concentration of 1 in 1000



**Figure 16** Positive control for p53 staining of cuff biopsy at concentration of 1 in 2000



**Figure 17** p53 positive control

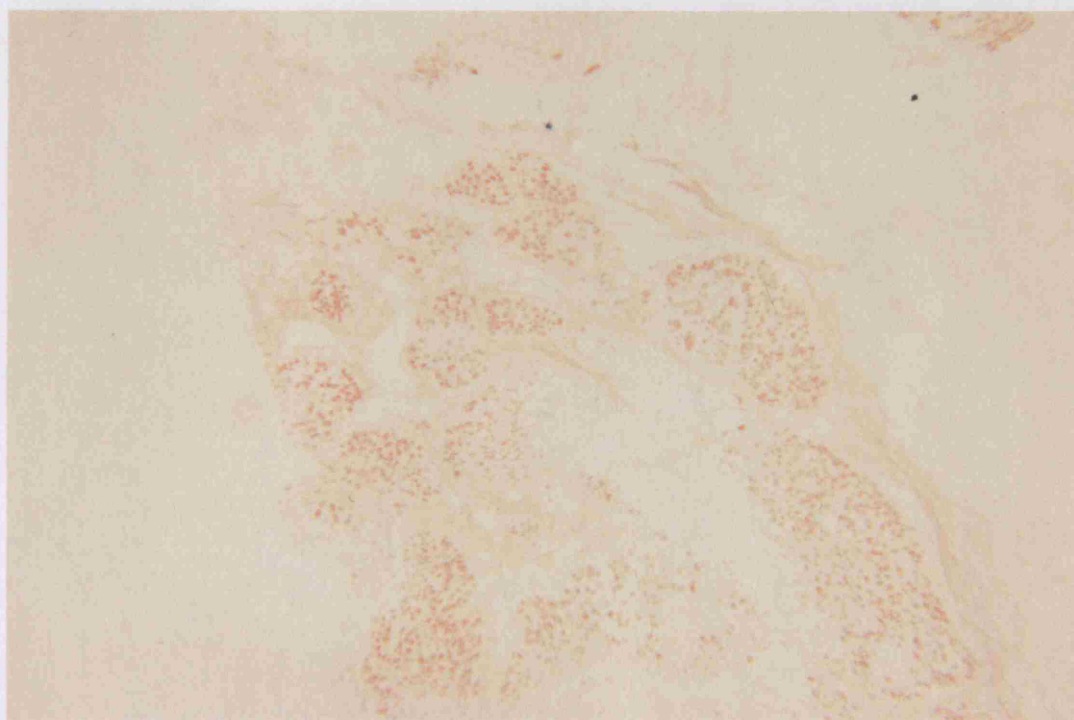
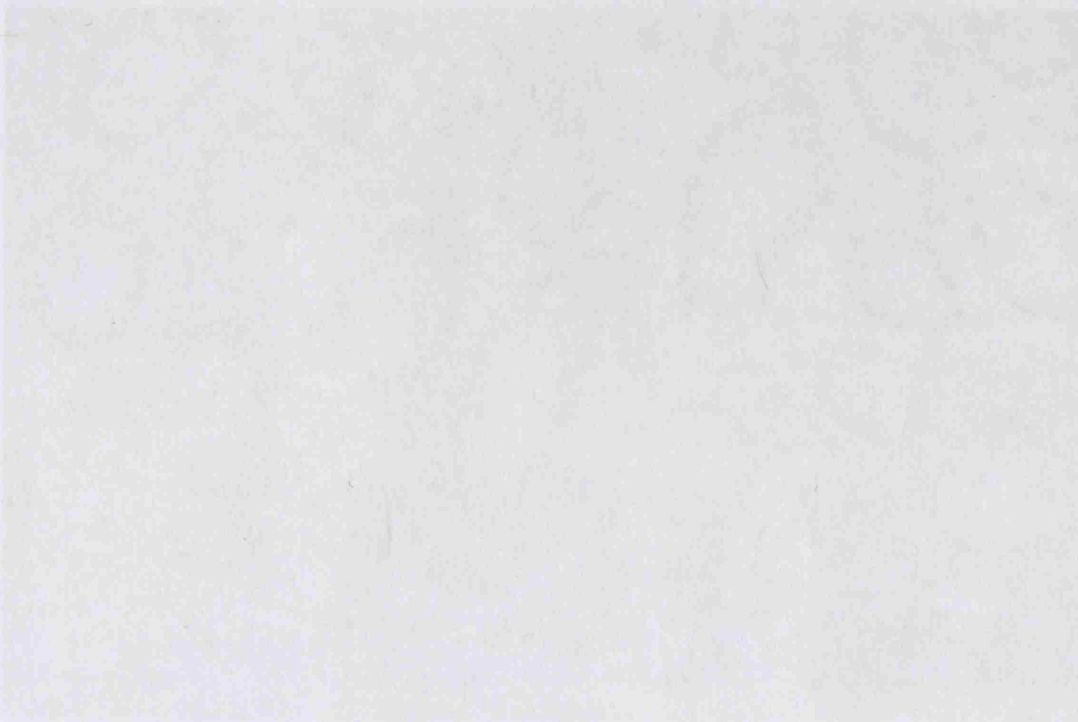
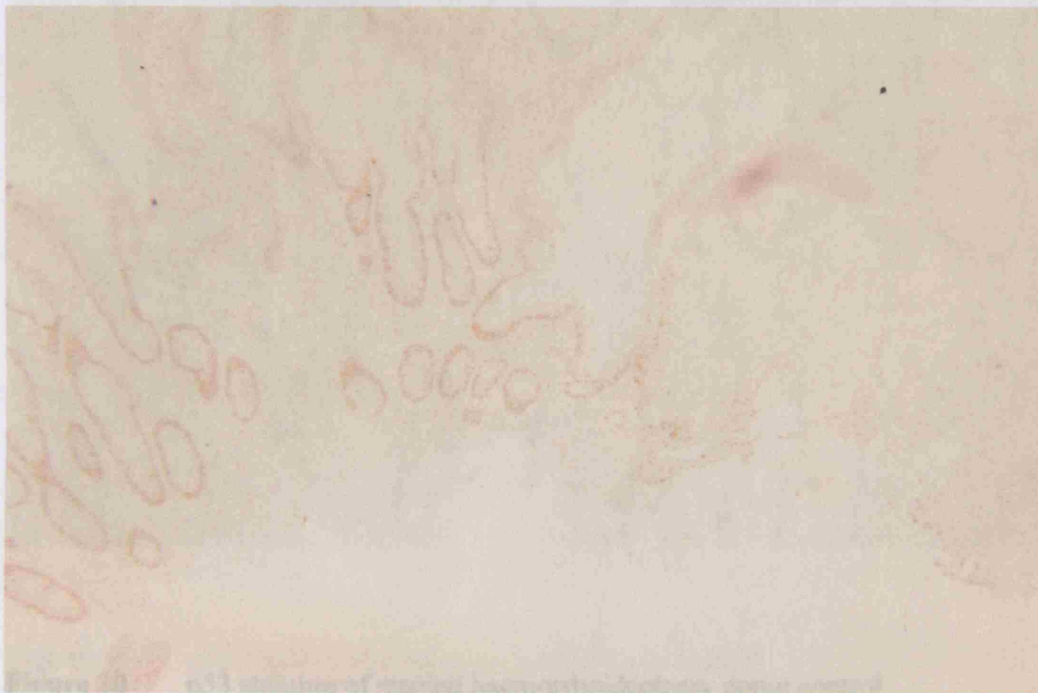
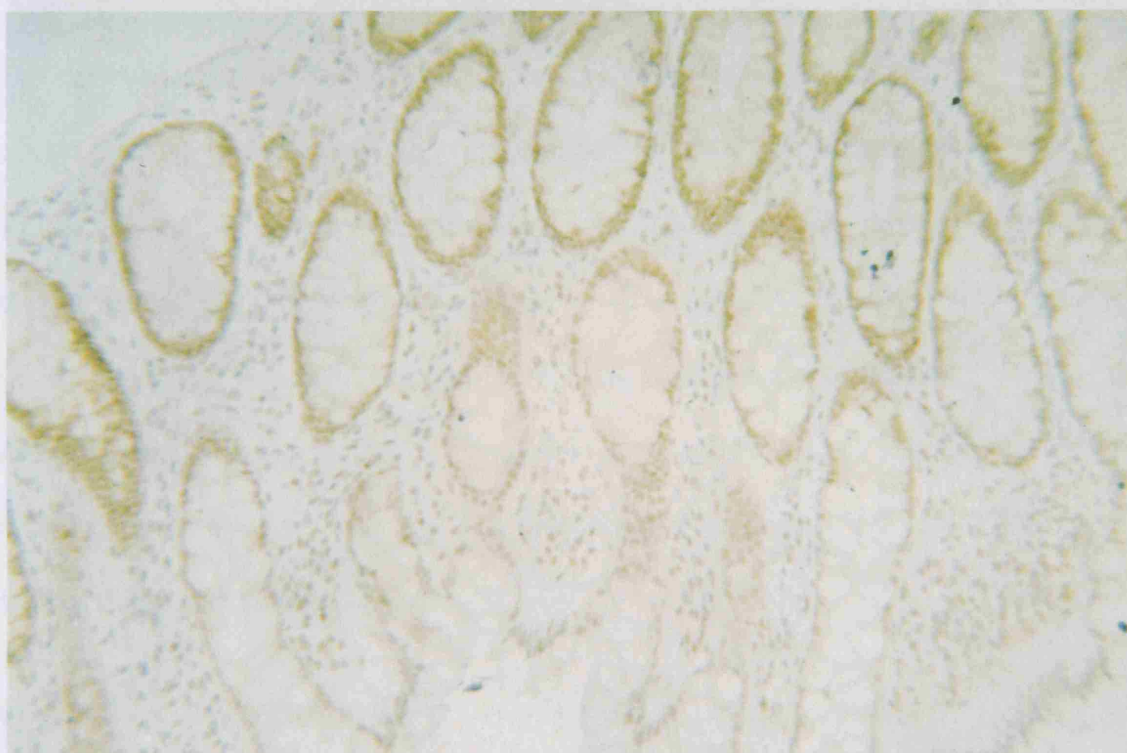


Figure 19 p53 staining of normal rectal biopsy control

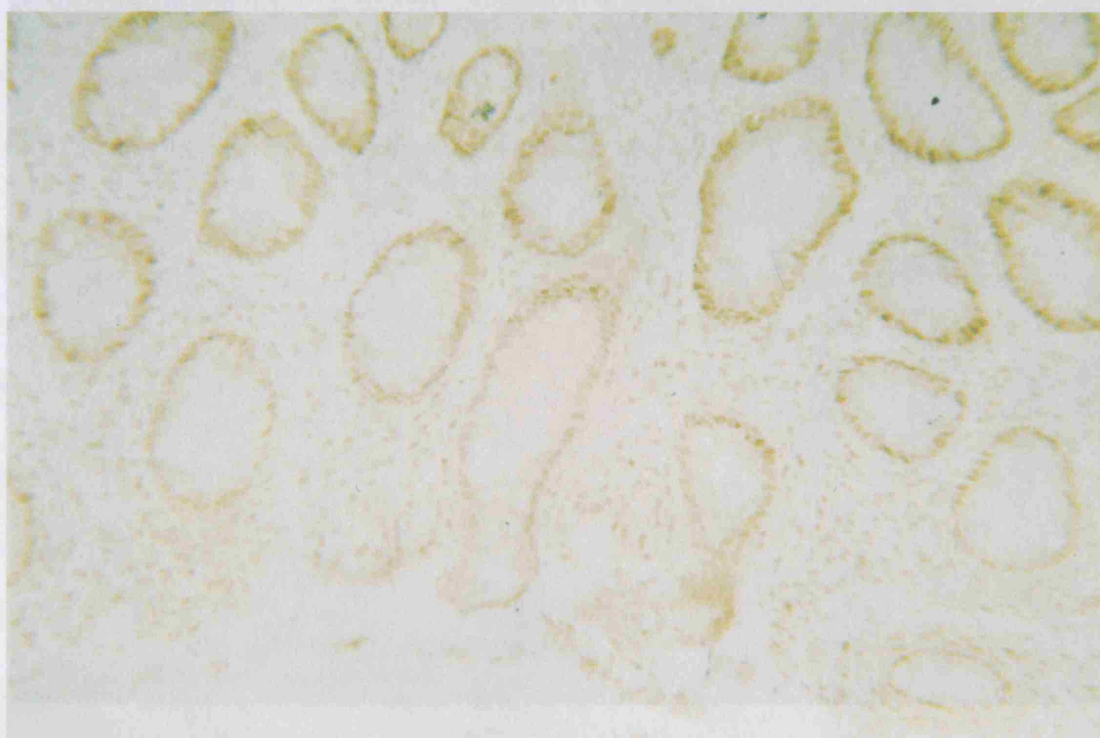
**Figure 18** Cuff biopsy staining positive for p53



**Figure 19** p53 staining of normal rectal biopsy control



**Figure 20** p53 staining of stapled haemorrhoidectomy donut control





**Figure 21** p53 staining of ileal pouch biopsy control



**Figure 22** p53 staining of ulcerative colitis biopsy control



Cuff (n=3)	0	0
Control	0	0
Rectum (n=3)	0	0
UC (n=3)	0	0
Pouch (n=3)	0	0
Donuts (n=3)	0	0

## 2.9 Results

Low grade dysplasia was present in three of the original 135 procto-colectomy specimens. None of these specimens showed evidence of high grade dysplasia or carcinoma. 173 cuff biopsies were attempted in the 110 patients who retained their pouch and agreed to undergo surveillance. Only 112 of these 173 biopsies (65%) from 75 separate patients, contained columnar epithelium from the rectal cuff; the remainder showed ileum or anal skin. The median elapsed time since the diagnosis of ulcerative colitis was 8.8 years (range 2-32) and the median elapsed time since pouch construction was 56 months (range 12-145).

None of the biopsies from the cuff showed dysplasia or carcinoma (0%).

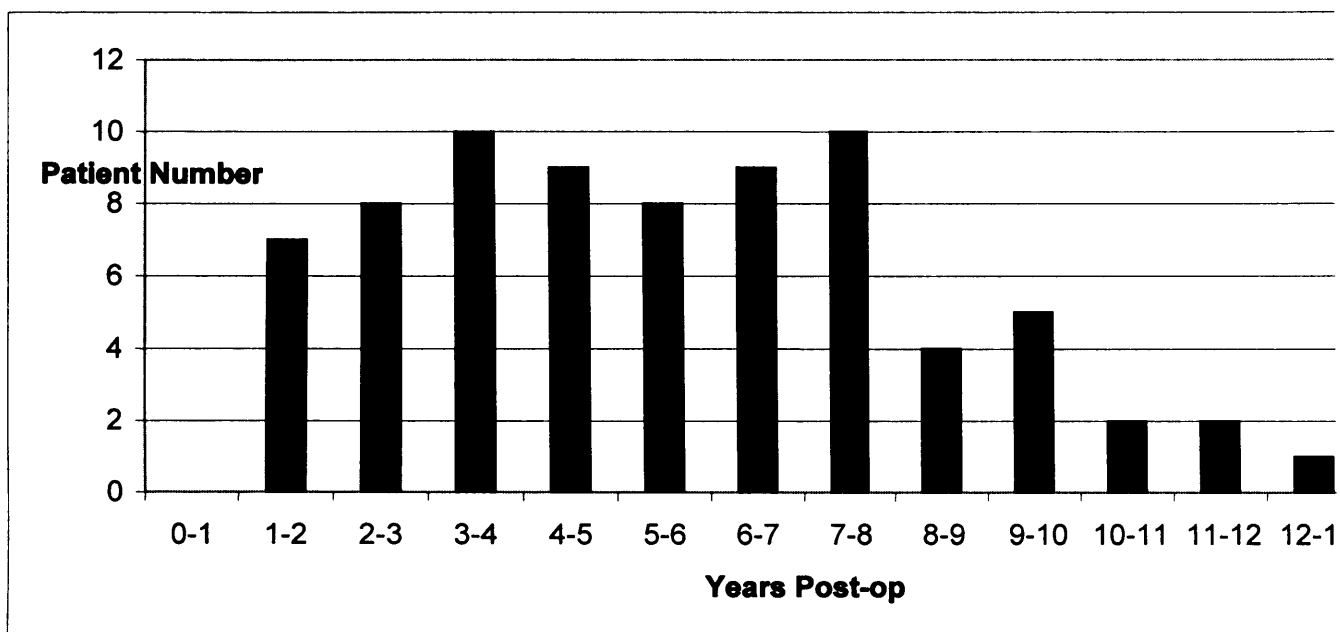
P53 overexpression occurred in 38 specimens (50.6%), but also occurred in many controls (3/3 normal rectum, 3/3 ulcerative colitis; 3/3 ileal pouch; and 6/6 stapled haemorrhoidectomy donuts). The results are summarised in **table 8**.

There was no correlation between p53 overexpression and ; degree of inflammation within the cuff biopsy, duration of UC, duration post-operatively, age or sex.

**Table 8** p53 expression in cuff biopsies and controls

	P53 +ve	p53 -ve
<b>Cuff(n=75)</b>	<b>38</b>	<b>37</b>
<u>Controls</u>		
Rectum(n=3)	3	0
UC(n=3)	3	0
Pouch(n=3)	3	0
Donuts(n=3)	3	0

**Figure 23** Number of patients with p53 immunostaining of cuff biopsies vs. time



## **2.10 Discussion - feasibility of using p53 staining of cuff biopsies as a pre-dysplastic marker following restorative proctocolectomy for ulcerative colitis.**

This study presents the first reported figures of p53 immunohistochemical staining of the columnar cuff following RP. The data is combined with our previously published figures of the second-longest reported follow-up of dysplasia in the rectal cuff after RP for UC (range 1-12yrs )<sup>201</sup>

The p53 suppressor gene is the most frequently altered gene in solid human malignancies. It is located on the short arm of chromosome 17 in the region 17p13 and encodes a 53kD nuclear phosphoprotein with specific DNA binding properties. P53 is a transcription factor which regulates cell growth and inhibits cells from entering S phase. Mutant p53 gene product lacks DNA binding capability and suppressor activity. In colorectal neoplasia, p53 dysfunction is important in the progression from adenomas into invasive carcinoma. Mutations of p53 in colorectal carcinomas are usually accompanied by loss of the other allele. Allelic deletion of 17p may have prognostic value in colorectal cancers. Therefore, mutation of p53 appears to be an important factor in the tumour biology of colorectal and other neoplasms.<sup>10</sup>

Mutation of p53 occurs most frequently in exons 5-9. Analysis of the mutations at the molecular level is not currently a routine procedure in pathology laboratories.

However, mutant p53 gene product is characterised by a conformational change of the protein with resultant prolonged half-life and stability. The accumulated mutant protein is therefore detectable in nuclei by immunohistochemistry.<sup>10</sup>

We observed no evidence of dysplasia, carcinoma or of abnormal stabilisation of p53. Furthermore, there was no apparent relationship between p53 stabilisation and dysplasia existed in these samples.



The development of colorectal cancer in patients who have UC has been widely described since the first report in 1925.<sup>204</sup> It is directly related to the duration and extent of the disease. The hypothetical risk of neoplastic transformation in the cuff, extrapolated from the available data for patients with UC who have had a IRA, has been calculated to be 1% at 30 years.<sup>36</sup> This would double to 2% if an additional 2 cm of rectal mucosa was inadvertently retained above the anal canal. Despite these low theoretical risk rates for malignant change in the rectal cuff, it has been considered necessary to follow patients using surveillance biopsies. Clearly, the discovery of a pre-dysplastic marker within the columnar cuff would allow the clinician to instigate more frequent surveillance and if necessary, perform a mucosectomy and pouch advancement.

In our previous study there was no evidence of either dysplasia or carcinoma in the rectal cuff at up to 12 years post pouch formation and up to 32 years post diagnosis of UC.

As detailed above in **table 7**, to date there have been eight published series from six centres, which report the risk of dysplasia and carcinoma in cuff biopsies.

<sup>36;177;181,180;205</sup> Although the follow up is short in most of these studies, taken together, only four patients had persistent evidence of dysplasia in the cuff.

Using dysplasia as the sole marker of cancer risk in cancer surveillance programs is problematic for several reasons. Both inter- and intra-observer variability are high, even among experts. Agreement on the diagnosis and degree of dysplasia is low, ranging from 42% to, at most, 65%.<sup>176;188</sup> Also dysplastic cells can be missed in areas of active inflammation, thereby decreasing sensitivity.

Despite the fact that some p53 point mutations escape detection by

immunohistochemistry, they are common in UC-associated dysplasia and cancer and this technique has advantages over molecular DNA assays that make it more suitable for clinical use, including ; technical accessibility, inexpensiveness, accommodation of small tissue samples, and it is associated with less inter-observer variability than dysplasia.<sup>185;197-199;201;206-208</sup> The efficacy of immunohistochemistry may be further enhanced by the development of other antibodies or antibody combinations directed against different p53 epitopes.<sup>209</sup> Nevertheless, the potential for p53 overexpression to occur despite the absence of p53 mutations, as previously reported in non-colorectal tissues, mandates caution in interpreting results based solely on this technique.

Both a mutation and loss of an allele (loss of heterozygosity) need to occur before p53 loses its function. Single strand conformation polymorphism (SSCP) and polymerase chain reaction (PCR) currently are the most precise methods for identifying p53 suppressor gene mutations and loss of heterozygosity but these techniques are currently too expensive and time-consuming for routine use in cancer surveillance.<sup>200</sup>

Several antibodies are commercially available for the immunohistochemical detection of p53 mutations. The monoclonal antibody DO-7 was employed, based on a previous study of six antibodies that showed DO-7 to have the highest sensitivity and specificity for the detection of genetic p53 mutations in colorectal cancer.<sup>10</sup>

Although we found that 38/75 (50.6%) of cuff biopsies stained positive for p53 overexpression, we cannot report any apparent abnormal stabilisation of p53 within the columnar cuff, since positive immunohistochemical results were also seen with the negative controls ; normal rectum, normal pouch, inflamed UC and stapled haemorrhoidectomy. These may have stained positive due to the rapid turnover of cells in these distal areas. Interestingly, the other studies quoted in the literature report

omitting the primary antibody as a negative control but none of the others describe using normal and other forms of intestinal tissue as controls, as we did, except Taylor et al. who used a less sensitive monoclonal antibody to p53, Pab1801, and found no positive immunoreactivity in their negative control of normal mucosa from resection margins of lengths of surgically removed colon..

There have been several reports of immunohistochemical positivity in non-colorectal issues without p53 mutations.<sup>48;210-213</sup> From a group of 95 patients with chronic UC, Lashner et al.<sup>200</sup> found positive p53 staining in 10 patients with no evidence of dysplasia in their biopsies. They reported that immunohistochemistry was 77% (27/35) sensitive for dysplasia or cancer and 83% (50/60) specific for dysplasia or cancer. They concluded that p53 mutations developed approximately eight months before low-grade dysplasia, 26 months before high-grade dysplasia and 38 months before cancer. Brentnall et al. studied UC-associated neoplastic progression and stated that they were the first to report p53 mutations in mucosa that was negative for dysplasia<sup>199</sup> with a frequency of 29%. They found the p53 mutation to be an early event in the process of UC-associated tumorigenesis and loss of heterozygosity to be a relatively late event. Harpaz et al. also used immunohistochemistry and found p53 overexpression in ; 34/56 (60.7%) UC-associated colorectal carcinomas, 16/20 (80%) dysplastic epithelia adjacent to carcinoma and 9/20 (45%) dysplastic masses remote from carcinoma.

They found independence of p53 overexpression, of the grade of dysplasia, suggesting that p53 inactivation in dysplastic epithelia in UC may be an independent marker of malignant potential. Holzmann et al. used SSCP on UC biopsies to identify p53 mutations in 40/212 (19%) without dysplasia, 17/29 (41%) with indefinite dysplasia, 9/24 (38%) with low grade dysplasia and 5/8 (63%) with high grade

dysplasia.<sup>198</sup>

The failure of immunohistochemistry to detect some tumours containing p53 that are identifiable by single strand conformation polymorphism analysis (SSCP) has been noted previously<sup>214</sup> and indicates that some mutations do not result in detectable overexpression of the p53 protein. Assessment of the value of p53 determination as an adjunct to conventional histopathological evaluation in the management of high risk UC patients awaits further investigation, including longitudinal studies of patients enrolled in endoscopic surveillance programs. Such studies might ascertain, for example whether p53 status influences the prognostic significance of biopsy specimens with low grade dysplasia.<sup>197</sup>

The immunohistochemical method employed to indicate probable p53 mutation may have missed some non-sense mutations resulting in a truncated protein. In addition, given the high degree of sensitivity of antigen retrieval methods, overexpression of wild type protein may have been detected i.e. appropriate expression of non-mutant p53.

It has been hypothesized that aneuploidy in biopsy specimens interpreted as indefinite for dysplasia may predict future progression to definite dysplasia: Rubin et al.

<sup>215</sup>presented a prospective study of 25 high risk patients without dysplasia, among whom 19 without aneuploidy did not progress to dysplasia in one to two and a half years but five out of six patients with aneuploidy developed dysplasia in the same time. Only one patient with aneuploidy did not develop dysplasia. Lofberg et al.

<sup>205</sup>described the same phenomenon: a prospective study of 59 patients with long-standing UC showed that DNA aneuploidy occurred in six patients before, and in another six simultaneously, with development of definite dysplasia. Only one patient developed dysplasia before DNA aneuploidy occurred. Holzmann et

al.<sup>216</sup> found that 35% of their patients in a similar group of patients, who had negative histology, also had aneuploidy.

If p53 testing of surveillance biopsies is combined with testing for dysplasia, two populations of patients with UC will be identified. Patients without dysplasia in the cuff who are p-53 positive could be considered at high risk could be considered at high risk for the development of carcinoma within the cuff.

If so, to reduce cancer mortality, such patients would need to undergo more frequent surveillance testing to detect dysplasia, or to be considered for pouch advancement and mucosectomy before cancer develops. Likewise, patients with low grade dysplasia but no p53 mutations may be at lower risk for cancer mortality than previously thought and perhaps such patients could delay further surgery until either p53 mutations or high grade dysplasia develop.

In conclusion, we found no evidence of dysplasia or carcinoma within cuff surveillance biopsies . Dysplasia is uncommon in cuff biopsies following RP for UC and has never persisted in the cuff of a patient whose original resection specimen showed no evidence of dysplasia or carcinoma. Whilst positive immunoreactivity is seen within half of cuff surveillance biopsies, no evidence of abnormal stabilisation of p53 could be reported. We conclude that p53 expression was not useful in surveillance of cuff biopsies from patients who have undergone RP for UC and the search should continue for alternative pre-dysplastic markers.

There was no apparent relationship between p53 stabilisation and dysplasia in these samples. These data suggest that cuff surveillance in the first decade after pouch formation is unnecessary. However, we consider regular cuff surveillance biopsies should continue for patients with high grade dysplasia or carcinoma in the original colectomy specimen.

**CHAPTER THREE**

**SEXUAL FUNCTION AND  
PREGNANCY RATES FOLLOWING  
RESTORATIVE  
PROCTOCOLECTOMY**

### 3.1 Introduction

Restorative proctocolectomy is frequently performed on young, sexually active, fertile adults diagnosed with UC or FAP because it avoids the need for a permanent stoma. Although RP improves self-image by eradicating the need for a permanent stoma, it may result in damage to autonomic nerves of sexual and bladder function during pelvic dissection. Consequently, male patients may suffer with erectile dysfunction, which may be complete (impotence) or partial. They may also suffer ejaculatory dysfunction in the form of retrograde ejaculation.<sup>217</sup> All of these factors may subsequently result in infertility. In females, the equivalent pelvic autonomic nerve damage may result in vaginal dryness and failure of excitation and orgasm.<sup>218</sup> RP may mechanically distort the female pelvic anatomy and lead to dyspareunia.<sup>96</sup> Anatomical distortion and post-operative adhesions are known causes of fallopian tube occlusion.<sup>96</sup> These factors may all potentially result in female infertility following RP.

Reduced libido and soiling during intercourse have also been previously reported.<sup>96</sup> After pouch surgery men should be able to father children and women should be able to fall pregnant and deliver safely by either caesarean section or per vaginum. Since there is a paucity of information regarding the effect of RP on sexual function, fertility and fecundity the results of a study to examine fertility rates before and after pouch surgery, and aspects of sexual dysfunction as causative factors for the discrepancy, are reported.

### 3.2 Patients and methods

A postal questionnaire was sent to 149 consecutive patients (84 males and 65 females) who underwent RP at our institution between 1988 and 2000 and had a functioning “J” pouch with at least 12 months follow. Patients less than 18 years of age were excluded. In all patients the operation involved the anastomosis of an ileal J- pouch to the upper anal canal using a “double staple” technique. There was no dissection in the anal canal and the transitional zone at the dentate line was preserved. A bloodless dissection of the rectum was undertaken using diathermy which allows the pelvic nerves to be identified and preserved.

The questionnaire was modified from those used in previous reports<sup>96,218-223</sup> with a different composition for men and women. (see **appendices ii and iii**).

Specific information was sought regarding various aspects of sexual function before and after surgery. Completion of the questionnaire was voluntary and anonymous. Patients could refuse to answer any questions.

### 3.3 Results

One hundred and twenty patients (81%) responded to the questionnaire; 79% of males and 83% of females. Six patients (four men) had the pouch formed for FAP; the remaining patients had UC. The median age of the patients was 41 years (19-67) for men and 38 years (range 20-61) for women. The median duration of follow up since formation of the pouch was 6 years (1-12) for men and 6 years (1-12) for women. The median time interval since diagnosis of the ulcerative colitis was 10 years (1-32) in men and 11 years (1-26) in women.



### 3.3.1 Male sexual dysfunction

Fourteen of the 66 males (21%) were sexually inactive after surgery. Seven men (12%) were unable to achieve orgasm during intercourse. The effect of surgery on male sexual function is shown in **table 9**.

**Table 9** The effect of pouch surgery on male sexual function

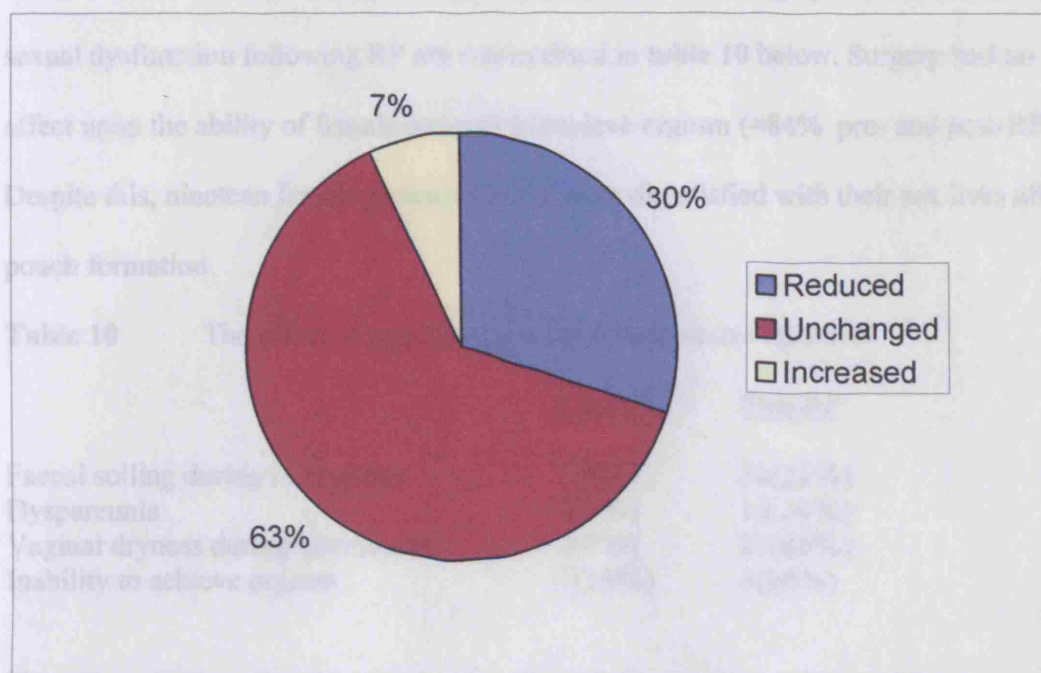
	<u>Pre-RP</u>	<u>Post-RP</u>
Inability to obtain erection	1( <b>1.5%</b> )	5( <b>8%</b> )
Difficulty in obtaining erection	1( <b>1.5%</b> )	18( <b>28%</b> )
Inability to sustain an erection	1( <b>1.5%</b> )	20( <b>32%</b> )
Painful erections	0	4( <b>6%</b> )
Inability to achieve orgasm	1( <b>1.5%</b> )	7( <b>12%</b> )
Inability to ejaculate	0	27( <b>44%</b> )
Faecal soiling during intercourse	4( <b>7%</b> )	9( <b>16%</b> )

Twenty patients (32%) complained of erectile dysfunction after surgery although only five (8%) were impotent - Table2. The most common abnormality was the loss of the ability to ejaculate (including retrograde ejaculation ) which occurred in twenty seven patients (44%).

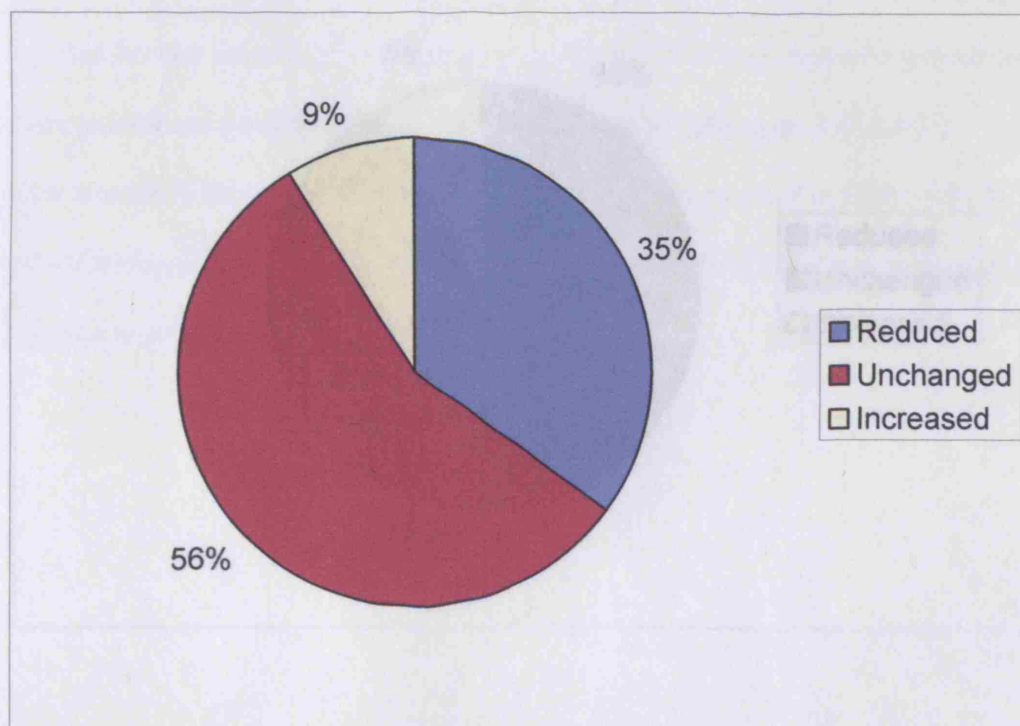
Overall, twenty seven men (44%) were dissatisfied with their sex lives after pouch formation and nine (16%) had suffered with faecal soiling during intercourse.

The effect of RP on quality of orgasm and the ability to achieve orgasm in males is depicted below in **figs. 24 and 25**.

**Figure 24** Comparison of the ability of male patients to achieve orgasm pre- and post-RP



**Figure 25** Comparison of the quality of male orgasm pre- and post-RP



### 3.3.2 Female Sexual Dysfunction

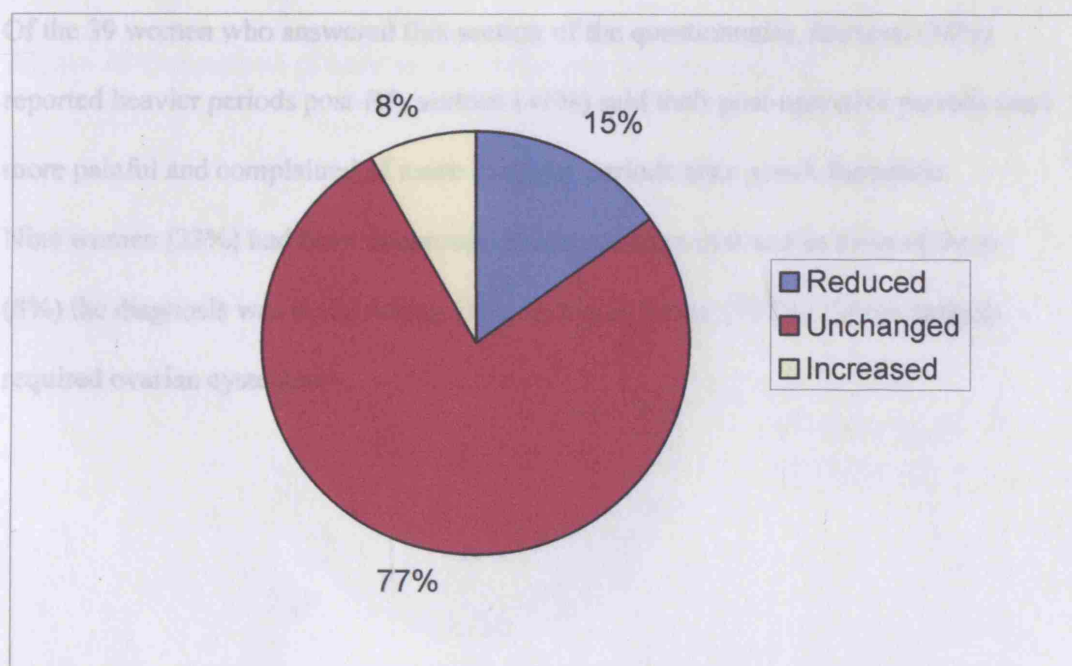
Seven of the 54 females (13%) were sexually inactive after surgery. Results for female sexual dysfunction following RP are summarised in **table 10** below. Surgery had no effect upon the ability of female patients to achieve orgasm ( $\approx 84\%$  pre- and post-RP). Despite this, nineteen female patients (40%), were dissatisfied with their sex lives after pouch formation.

**Table 10** The effect of pouch surgery on female sexual function

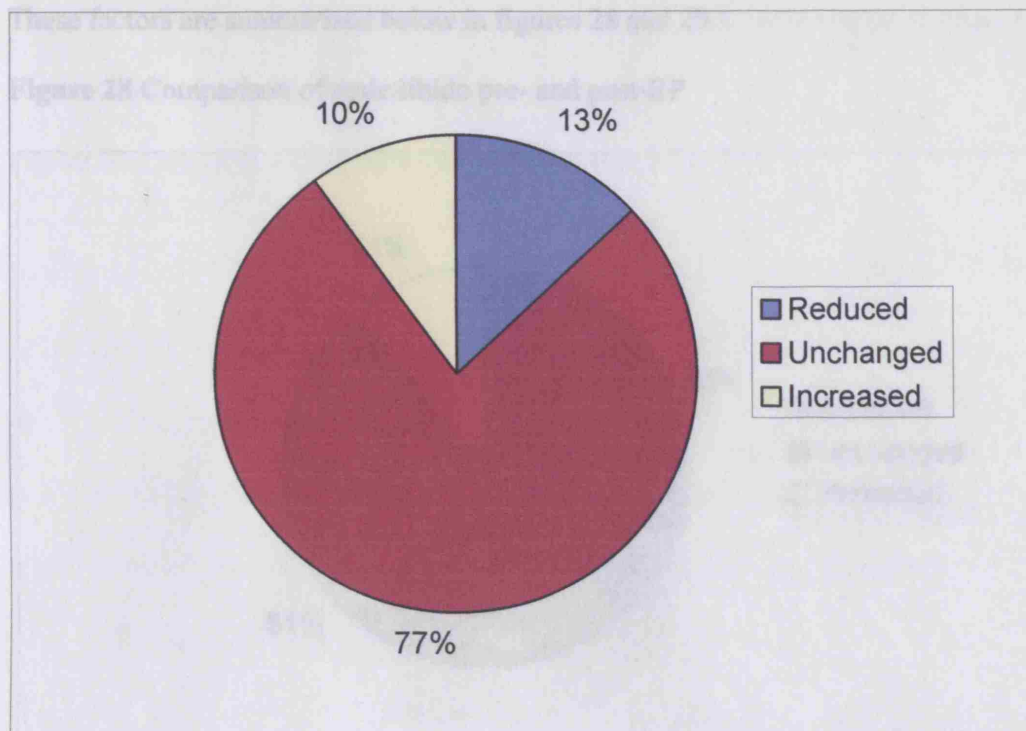
	<u>Pre-RP</u>	<u>Post-RP</u>
Faecal soiling during intercourse	3(7%)	10(21%)
Dyspareunia	2(5%)	16(36%)
Vaginal dryness during intercourse	3(7%)	21(46%)
Inability to achieve orgasm	7(15%)	8(16%)

The effect of RP on female orgasms is depicted below in figures 26 and 27

**Figure 26** Comparison of the ability of female patients to achieve orgasm pre- and post-RP



**Figure 27** Comparison of the quality of female orgasm pre- and post-RP



### 3.3.3 Menstrual irregularities

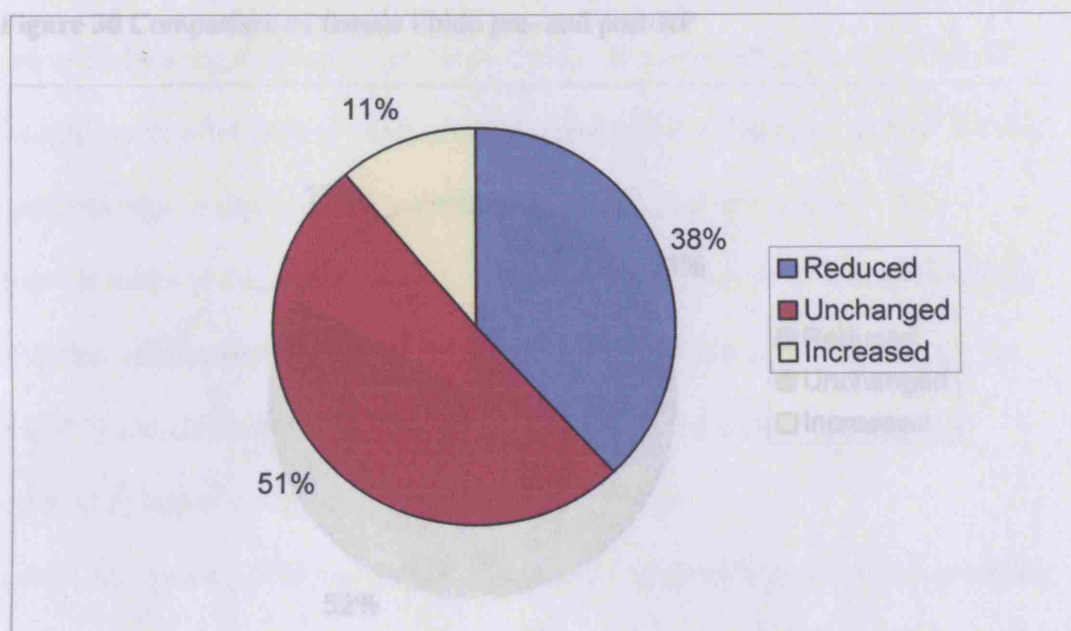
Of the 39 women who answered this section of the questionnaire, fourteen (36%) reported heavier periods post-RP, sixteen (41%) said their post-operative periods were more painful and complained of more irregular periods after pouch formation.

Nine women (23%) had been diagnosed with an ovarian cyst and in three of these (8%) the diagnosis was made during IVF treatment. Seven (18%) of these patients required ovarian cystectomy.

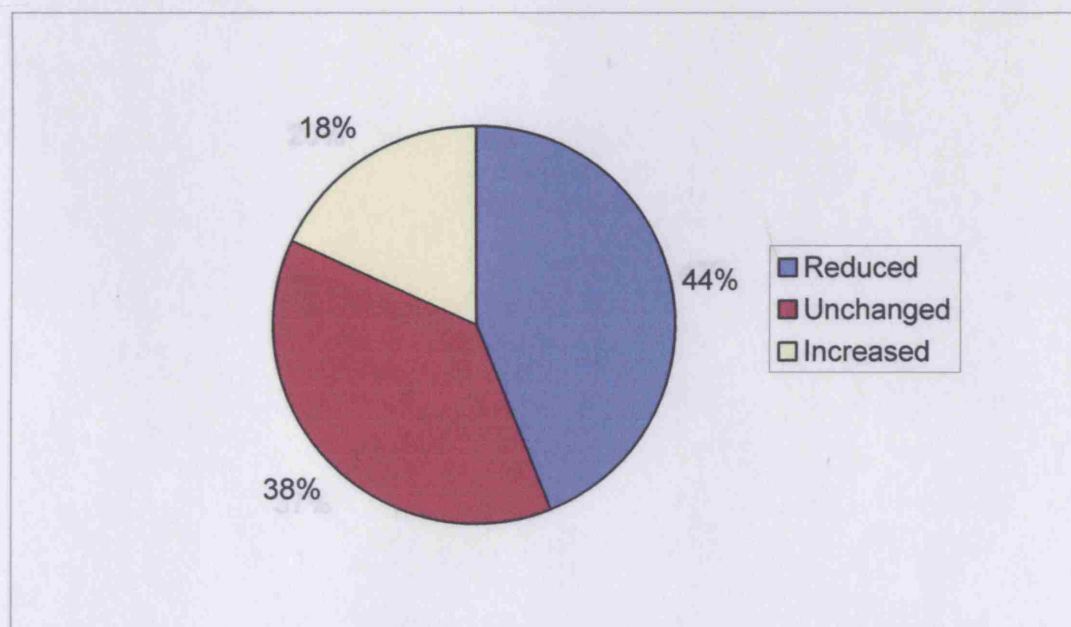
### 3.3.4 Male sexual frequency and libido

These factors are summarised below in figures 28 and 29

**Figure 28** Comparison of male libido pre- and post-RP



**Figure 29** Comparison of male sexual frequency pre- and post-RP

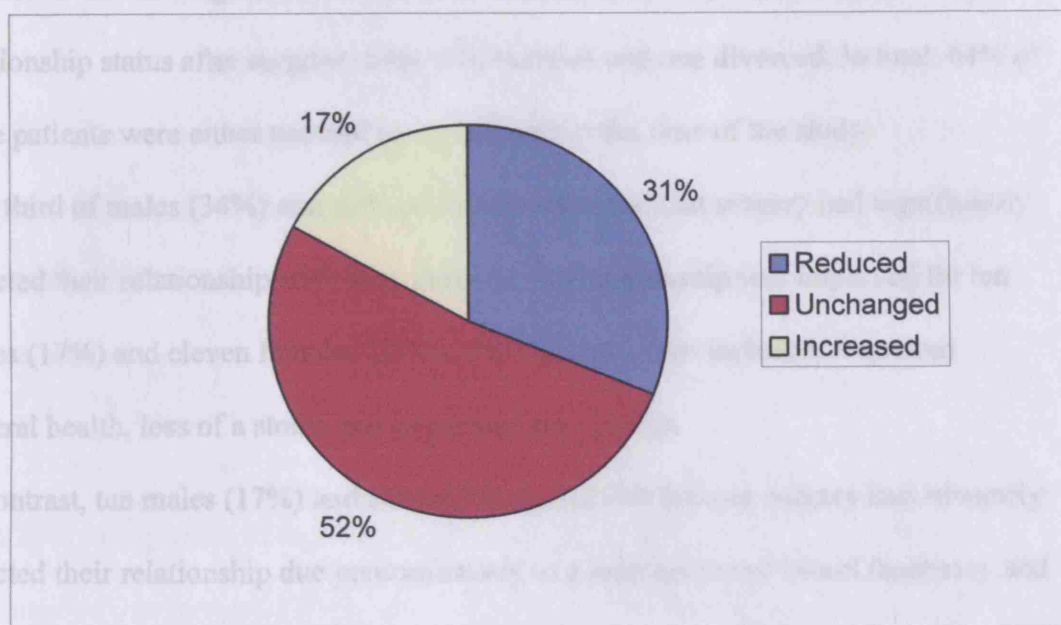




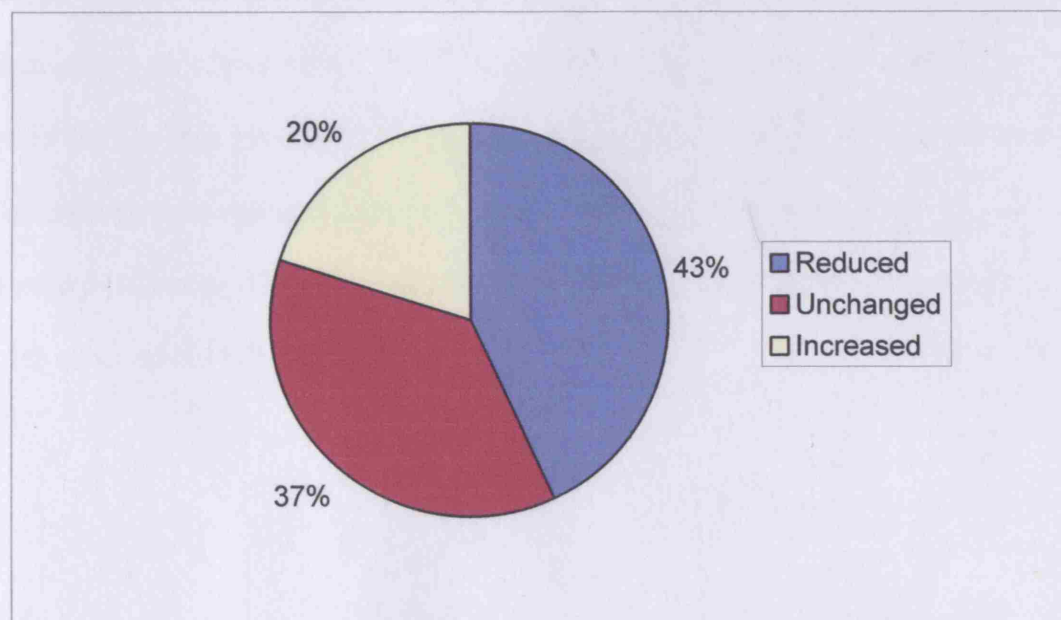
### 3.3.5 Female sexual frequency and libido

The effect of surgery on female sexual frequency and libido were similar to those found in men as seen in figures 30 and 31.

**Figure 30** Comparison of female libido pre- and post-RP



**Figure 31** Comparison of male sexual frequency pre- and post-RP



### **3.3.6 Association of RP with marital status and relationships**

Eleven female patients (20%) changed relationship status after surgery; Six (11%) married and two (4%) divorced. Overall, thirty eight female patients (70%) were married or co-habiting at the time of the study. Seven male patients (11%) changed relationship status after surgery; four (6%) married and one divorced. In total, 64% of male patients were either married or co-habiting at the time of the study.

One third of males (34%) and 42% of females reported that surgery had significantly affected their relationship with their partner. The relationship was improved for ten males (17%) and eleven females (21%). The reasons given included; improved general health, loss of a stoma and improved self- image.

In contrast, ten males (17%) and eleven females (21%) thought surgery had adversely affected their relationship due predominantly to a high nocturnal bowel frequency and faecal leakage especially during intercourse.

### **3.3.7 Pregnancy rates of partners of male patients who have undergone RP**

For the responding male patients, a documented pregnancy was recorded in the partners of 37 patients (**56%**) before SRP (89 pregnancies in total) and six patients (**9%**) after SRP (10 pregnancies in total). Thirteen of the 66 responding patients (**20%**) tried to impregnate their partners after SRP with at least twelve months unprotected intercourse and six were successful (**46% fertility**). Only one male patient, who had a low sperm count, tried IVF and this proved unsuccessful. Only one male patient (who had a low sperm count) tried in vitro fertilisation and this proved unsuccessful.

### **3.3.8 Pregnancy rates of female patients following RP**

There were 76 pregnancies from 32 women prior to pouch surgery, but post-operatively this number fell to 4 pregnancies from 3 women. All four babies were delivered by caesarean section, three on the advice of the patients' colorectal surgeon and one as a result of patient preference. Of the twelve women who had tried to fall pregnant from at least twelve months unprotected intercourse following RP, only two (**17% fertility**) were conventionally successful and a third fell pregnant with the aid of *in vitro fertilisation (IVF)*. In total only one of seven (14%) females who tried IVF post-RP successfully fell pregnant.



### 3.4 Discussion

UC and FAP patients frequently undergo surgery during their reproductive years,<sup>55</sup> when sexual contacts are most commonly established. Poad and Arnold<sup>224</sup> reported that patients identified the lack of information about the potential effects of surgery on sexual function and fecundity as a major deficiency of pre-operative counselling.

Iatrogenic injury to the autonomic nerves of sexual function is an undisputed complication of proctectomy,<sup>217;225-227</sup> but there is still a paucity of information documenting the resultant sexual dysfunction.

Erection is dependent upon intact S2-4 parasympathetic nerve supply through pelvic splanchnic nerves to the penis. Normal ejaculation is an L1+2 mediated sympathetic response via the hypogastric plexus that constricts the bladder neck forcing semen forward into the urethra. Male sexual dysfunction following RP is sparsely documented and has ranged from two per cent to 15% in previous studies.<sup>228-230</sup>

Comparative results are seen in **table 11**.

**Table 11** Male sexual function and fecundity following RP

<u>Author</u>	<u>Year</u>	<u>n</u>	<u>F.U.</u>	<u>impotence</u>	<u>orgasm</u>	<u>soiling</u>	<u>libido</u>	<u>fecundity</u>
Damgaard	1995	26	100%	4%	↓4%	-	same	-
Tiainen	1999	44	86%	7%	-	↓	same	-
Lindsey	2001	162	81%	4%	-	-	-	-
Present	2004	66	79%	8%	↓30%	16%	↓38%	46%

The majority of our patients (88%) were able to achieve orgasm after RP.

However we revealed relatively high figures for soiling during intercourse, (16%)

Inability to ejaculate (44%) and erectile dysfunction. (32%) We also observed a

reduction in sexual frequency in 44% of our patients and reduced quality of orgasm in 35% after RP. The inability to ejaculate was noticeably more common in our series compared with other publications.<sup>222;229</sup> Whether these results relate to the technical performance of RP in our hands is uncertain. Previous research has failed to show that close rectal dissection is associated with better male sexual function than is experienced after rectal excision in the mesorectal plane.<sup>229</sup>

Radiological hysterosalpinography and vaginography of women who had undergone RP, showed dorsal displacement and fixation of the fornix of the vagina, which was markedly dilated, and adherence of the fallopian tubes to the bottom of the lesser pelvis<sup>231</sup>. The position of the vagina and its angulation was unaltered compared to normal controls<sup>231;232</sup> suggesting that the interposed pouch prevents its dorsal displacement. Such anatomical alterations combined with sphincter dysfunction and damage to the autonomic pelvic plexus may account for the sexual dysfunction seen in this study.

Previous studies of panproctocolectomy and ileostomy<sup>221</sup> show dyspareunia rates of 30%, probably due to the alteration of vaginal anatomy. The rate of female post-operative dyspareunia in our study was slightly higher at 36% than the 0-33% previously reported.<sup>13;55;128;222;233-235</sup>

Soiling during intercourse previously reported in 0-15%,<sup>96;218;219;222</sup> affected 21% of our female patients.

Vaginal lubrication during intercourse depends on intact pelvic parasympathetic nerve supply, sexual arousal and menopausal status. There is very little documentation of the incidence of vaginal dryness following RP but Bambrick *et al.*<sup>223</sup> reported that 27% of their patients had increased vaginal dryness post-operatively and in one other

study,<sup>218</sup> 23% of women complained of this symptom. The response rate in the former study was only 35% which may explain why we found that 46% of women suffered with vaginal dryness post-RP from a response rate of 83%.

Increased sexual frequency in 22% and 35% of women after surgery has been reported in two other studies<sup>222;223</sup> similar to 20% in our group. One of these studies found that no women had reduced sexual frequency post-operatively,<sup>222</sup> whilst the other reported a reduction in 18%,<sup>223</sup> significantly less than the 43% of women who had sex less frequently after RP, in our study. Whilst half our female patients noted no change in their post-operative libido, 31% reported a reduced libido, compared to 18-25% in other studies.<sup>96;223</sup>

Overall 70% of women were married or co-habiting in stable relationships at the time of the study and whilst one fifth stated that pouch surgery had improved their relationship with their partner, the same percentage (21%) thought that surgery had adversely affected their relationship.

Any change in fecundity after RP is an important consideration. Several publications have addressed the issue of fertility and fecundity amongst female patients undergoing RP.<sup>96;219;220;223;218</sup> However, we are not aware of any reports of the relationship between RP and male fecundity and fertility.

In the present series fecundity was reduced following RP; pregnancy occurred in the partners of fewer than half of the 13 male patients who were attempting to conceive after SRP. Fecundity refers to the physiological capability of producing a live-born child.<sup>220</sup> In contrast, the term fertility is used to refer to the childbearing performance of a couple and therefore depends upon intention to conceive as well as ability.<sup>220</sup> In this study, pre-RP *fertility* was 56%, therefore pre-operative *fecundity* was *at least* 56%. It was not possible to quantify accurately the reduction in fecundity compared

with the pre-operative status since the proportion of patients who wished to conceive prior to SRP was not known. Although the data demonstrate a relationship between SRP and reduced fecundity they do not prove a causative association. Clearly the post-RP results are obtained from a shorter period of attempted conception with patients being older than they were before pouch formation. Other factors, for example the health of a patient's partner or increasing age, may be confounding variables. The results of postal questionnaire surveys may be confounded by the unrecorded experiences of non-responders that may not mirror those of responders. Furthermore, such retrospective data collection relies on the patients' memory and honesty. Nevertheless, it seems reasonable to attribute reduced fecundity, at least in part, to the documented reduction in libido and sexual frequency after SRP. In turn these factors may have been secondary to post-operative sexual dysfunction including soiling during intercourse, impotence and retrograde ejaculation.

This retrospective cohort survey has shown that RP in male patients is associated with a significant incidence of infertility that may be related to post-operative sexual dysfunction. These potential complications should be discussed with patients and their partners prior to RP. Confirmation of the present data by a prospective study with comprehensive follow-up of a larger sample would be an informative exercise that could enhance patient counselling. The merits of pre-emptive measures to assist conception, for example semen storage, might be explored in these patients.

Previous studies suggest female fertility is unaffected by UC<sup>28;44;236;237</sup> and as in the general population, 80-90% of women with UC are able to become pregnant. The first report of a successful pregnancy and delivery following RP was in 1984<sup>238</sup> but there is still a paucity of data from large studies concerning this issue.<sup>219;220;230;233;239</sup>

It has already been documented that pregnancy and childbirth are safe in patients following RP.<sup>219;233;235;238;240</sup>

Regarding the safest method of delivery, caesarean section decreases the risk of incontinence and potential damage to the anal sphincters and pouch but is associated with all the complications of abdominal surgery, including formation of adhesions. Conversely, vaginal delivery may damage the pudendal nerve and anal sphincter mechanism.<sup>239</sup>

Some studies have suggested that fertility is unaffected in women following RP,<sup>233;241</sup> but other studies have not reached the same conclusion. Olsen et al.<sup>220</sup> reported 34 post-operative births from 14% of a group of 237 women and this figure represented 49% of the expected number of births in the population. Subtracting those who fell pregnant after IVF, the birth rate fell to 35% of the expected figure. They also revealed 29% of their pregnancies occurred after IVF as opposed to 1% of pregnancies in the normal population. An infertility rate of 20-93% post-RP was found in other studies. Post-operative births were only seen in 4-9% of female patients in other large studies,<sup>219;239;240</sup> reinforcing the apparent reduction in fertility.

In our own group of patients the low pregnancy rates post-RP may partly be explained by the reduction in libido and sexual frequency seen in both males and females, which in turn may have been secondary to increased male and female sexual dysfunction post-operatively such as erectile dysfunction, retrograde ejaculation, soiling during intercourse and dyspareunia – as detailed above.

Other than reduced sexual frequency post-RP the other most likely factor contributing to reduced post-operative pregnancy rates is the distortion of vaginal anatomy and effect of adhesions following pelvic surgery. Oresland et al.<sup>96</sup> showed with

hysterosalpinography and vaginography that; 10/21 post-RP females had fallopian tubes attached to the bottom of the lesser pelvis, 9/21 had unilateral tubal occlusion and two had bilateral tubal occlusion. They recommended that the adnexae be protected in temporary peritoneal pouches during surgery. Instillation of bioresorbable membranes has been reported to prevent formation of adhesions,<sup>242</sup> so these substances may have a role to play in improving fertility post-RP. One group<sup>219</sup> suggested that consideration be given to delaying pouch-anal anastomosis in patients who have already undergone colectomy and wish to bear children.

The incidence of ovarian cysts in the general population is 1-10 in 10,000 depending on the use of the oral contraceptive pill.<sup>243</sup> Our rate of 23% was higher than the 11% reported in another study.<sup>223</sup> For one third of our female pouch patients with an ovarian cyst, the diagnosis was made during IVF treatment. A further study by Counihan et al.<sup>219</sup> reported a 7.4% incidence of female RP patients with pelvic cysts.

We conclude that within our series low pregnancy rates were seen following RP and this was associated with a high incidence of male and female sexual dysfunction. These possible outcomes and available treatments should be discussed with patients prior to obtaining patient consent for pouch surgery.

**CHAPTER FOUR**  
**LONG-TERM HAEMATOLOGICAL**  
**OUTCOME FOLLOWING**  
**RESTORATIVE**  
**PROCTOCOLECTOMY**

#### **4.1 Introduction**

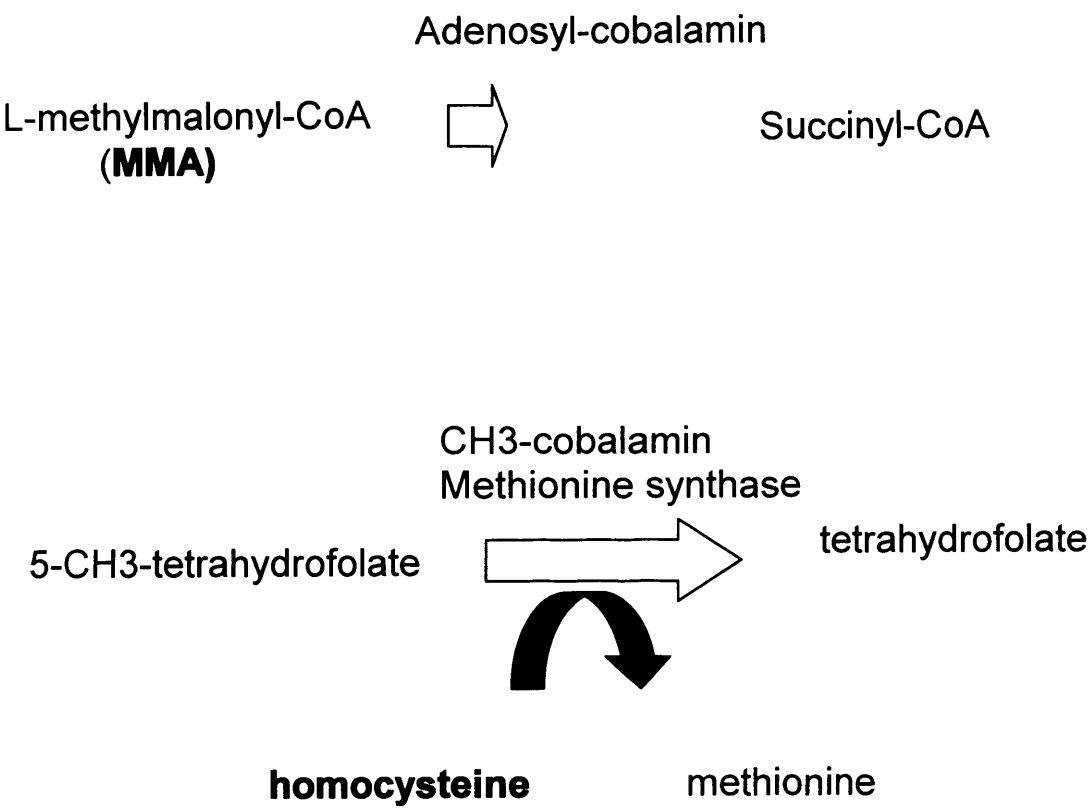
Restorative proctocolectomy is the preferred surgical procedure for patients with UC, a condition characterised by rectal bleeding and bloody diarrhoea, which may progress to anaemia. This relatively new procedure involves resection of the terminal ileum - the site of vitamin B12 absorption. The “pouch”, by design, leads to stasis of small bowel content and predisposes to bacterial overgrowth in both the ileal reservoir and the more proximal ileum and such overgrowth is known to predispose to vitamin B12 deficiency. Despite this effect, there is little information regarding the long term haematological and nutritional consequences of ileo-anal pouch surgery. It might be anticipated however that these patients will be at risk of developing vitamin B12 deficiency.

Vitamin B12 is the generic name for a group of cobalt-containing corrinoids with a biological activity in humans. Human serum not only contains the active cobalamins (mainly the hydroxo-, methyl- and 5'-deoxyadenosyl-cobalamins) but also a variable number of vitamin B12 analogues which are biologically inactive non-cobalamin corrinoids. Cyanocobalamin is normally not present in humans, except as very small amounts in smokers.

Vitamin B12 functions primarily as a coenzyme in intermediary metabolism, especially in transmethylation reactions. Only two vitamin B12 –dependent reactions have been identified in humans; the thymidylate synthetase reaction (DNA synthesis) and the methionine synthase reaction with methylcobalamin and 5-deoxyadenosylcobalamin as the active coenzymes.



**Figure 32**     The two cobalamin-dependent enzymes in human cells



Vitamin B12 is protein-bound in food and is separated from the protein complex by pepsin in the presence of stomach acid. It is then bound by intrinsic factor and transported from the proximal ileum to the distal ileum, where it is then bound to transcobalamin II for transport to the liver and bone marrow.<sup>244</sup>

The total body pool for an apparently healthy, omnivorous adult has been estimated to be between 2-2.5mg of which about 80% is contained in the liver: the daily loss of vitamin B12 is roughly 0.05-0.2% of the body pool, regardless of the pool size.

Deficiency of the vitamin in humans is manifested by the occurrence of megaloblastic anaemias and neurological disorders. The most common presenting neurological

symptoms are paraesthesia or ataxia, and the most common objective findings are diminished vibratory sensation and proprioception in the lower extremities. A variety of neuropsychiatric symptoms and signs have been described, including loss of cutaneous sensation, muscle weakness, diminished or hyperactive reflexes, spasticity, urinary or faecal incontinence, orthostatic hypotension, loss of vision, dementia, psychoses and disturbances of mood.<sup>34</sup> If patients with neuropsychiatric symptoms secondary to vitamin B12 deficiency do not have haematological abnormalities, treatment might be delayed and symptoms progress irreversibly.<sup>39</sup>

#### 4.2 Patients and methods

One hundred and ninety four patients underwent RP between 1988-2000. Haematology samples including ; full blood count, serum vitamin B12, serum and red cell folate and serum ferritin were obtained from all patients (100%), pre- and post-operatively. Eighty one patients were female (42%).

Indications for surgery :	Ulcerative Colitis	169 patients
	Indeterminate Colitis	5 patients
	Familial Polyposis	14 patients
	Slow Transit Constipation	6 patients

In those patients with UC the median duration of the disease prior to surgery was 11.2 years (range 0-32). The median age of all patients at last follow up was 40 years (13-67) and the median time interval since pouch surgery was 5.4 years (1-12). The surgical procedure used has been described before.<sup>162</sup> All patients had a stapled J-pouch fashioned with a minimum limb length of 15 cm. The pouch was stapled to the anal canal using a double stapled technique in all but six patients who underwent mucosectomy with a hand sewn anastomosis. The median length of ileum excised was obtained from the pathology report and was 3.1 cm (range 0.5-15 cm). Only six

patients did not have a temporary loop ileostomy fashioned at the time of formation of the pouch.

On all prospective patients, full blood count and haematinics were measured ; prior to colectomy, prior to pouch formation and prior to ileostomy reversal.

Post-operative patients in the study were offered routine follow up that included outpatient review at six weeks, six months, twelve months and annually thereafter. At each out-patient visit blood was obtained for a complete haematological assessment including : (normal range in brackets); haemoglobin (Male 13-18g/dl, Female 11.5-16.5g/dl), serum ferritin (6.9-283 µg/l), serum folate (3.5-16 ng/ml), red cell folate (180-613ng/ml) and serum vitamin B12 (188-1059 pg/ml). All available retrospective results from the hospital haematology database were also collated.

#### **4.2.1 Laboratory methods of haematological measurements of blood samples**

Full blood counts (FBCs) were measured by a Coulter GenS automated FBC analyser.

A microparticle enzyme immunoassay method (Abbott Diagnostics) was used to measure serum vitamin B12, folate and ferritin levels.

Patients with a subnormal serum vitamin B12 level subsequently underwent a Schilling test to examine B12 absorption across the terminal ileum and a hydrogen breath test to establish any evidence of bacterial overgrowth. They were also questioned for other known causes of vitamin B12 deficiency including : pregnancy and breast-feeding, vegan/vegetarian diets, coeliac disease, excessive alcohol intake, chronic pancreatitis, metformin or proton-pump inhibitor therapy, previous gastric surgery and a history of atrophic gastritis, achlorhydria or *helicobacter pylori* infection

#### **4.2.2 Schilling test for vitamin B12 deficiency**

After fasting overnight, the patient was given a capsule containing 1 µg oral cyanocobalamin labelled with 18.5 kBq of radioactive cobalt  $^{57}\text{Co}$ . Two hours later 1000 µg of non-radioactive hydroxocobalamin B12 was given intra-muscularly as a “flushing-out” dose and subsequently a 24-hour urine collection was performed. Radioactivity of the urine was compared to a standard and the percentage of the dose excreted in the urine was calculated. Normal urinary excretion to exclude malabsorption is greater than 10% of the oral test dose. In the event of failure of B12 absorption, a smaller percentage of B12 would be absorbed into the bloodstream and subsequently, less would be excreted in the urine.

#### **4.2.3. Hydrogen breath test for bacterial overgrowth**

Hydrogen is normally only produced in the colon when carbohydrates and glycoproteins are metabolised by bacteria. Following oral administration of a sugar, patients with small bowel contamination have an early peak of breath hydrogen that is produced by bacteria in the small bowel. After fasting overnight, two basal breath samples were measured for hydrogen levels. The patient then drank 50g of glucose dissolved in 250mls of tap water and duplicate breath samples were then taken every 30 minutes for a two hour period. A positive test for small bowel bacterial overgrowth was defined by a rise in breath hydrogen of more than 12 parts per million over the basal reading at any time during the two hour period

### 4.3 Results

Seventy one (37%) of the one hundred and ninety four patients, underwent an emergency subtotal colectomy prior to elective pouch formation. Full blood count results obtained from 64 emergency patients prior to colectomy showed 37 (58%) were anaemic compared to 53 (44%) of the 120 non-emergency patients with available results. Overall 171 patients (88%) were anaemic at some stage, comprising 73 females (43%) and 98 males (57%). Forty six (27%) of the patients with a low haemoglobin had a microcytosis ( $MCV < 76\text{fl}$ ) and three (2%) had a macrocytosis ( $MCV > 99\text{fl}$ ). Fifty three of the anaemic patients had a simultaneous sample analysed for serum ferritin and the level was abnormally low in 22 of them (42%) and normal in the others.

Results from 64 patients with an end-ileostomy following emergency surgery showed 19 (30%) were anaemic, in comparison to 40 of 106 patients (38%) with a loop ileostomy following elective pouch formation. After reversal of defunctioning ileostomy, Full blood counts were obtained from 168 patients, including 72 females (43%). Fifty nine of these patients (35%) were anaemic comprising 23/72 females (32%), and 36/96 males (38%).

Forty two (25%) of the 171 patients in whom vitamin B12 levels were measured had developed a sub-normal level, four (9.5%) pre-operatively and thirty eight (90.5%) post-operatively. None of these patients had any recognised risk factors for B12 deficiency listed above. Of these 42 patients, twenty two were female (53%). Thirty six of the patients with B12 deficiency had their pouch formed for UC, two for indeterminate colitis, three for FAP and one for Slow Transit Constipation (STC). The median time interval from pouch construction to the first detection of a sub-normal vitamin B12 was 2.9 years (0-9). In sixty-eight patients (73%) three or more vitamin

B12 measurements were obtained during follow up. Of these, 50 patients had a progressive decline in serum vitamin B12 although only 18 were sub normal.

Thirty six patients with a low serum B12 had a Schilling test performed. This was normal in 34 patients (94%), abnormal in one patient and equivocal in the remaining patient.

Thirty-six patients with low vitamin B12 levels had a hydrogen breath test performed to examine for bacterial overgrowth in the small bowel. One patient who did not produce hydrogen was excluded. Thirty four of the remaining thirty-five patients (94%) had a normal test and one patient had a positive test for bacterial overgrowth.

Until recently, vitamin B12 replacement therapy was available only by intra-muscular injection of hydroxocobalamin. Cyanocobalamin is now available in tablet form permitting oral replacement. Those patients with vitamin B12 deficiency and a normal Schilling test were considered suitable for oral replacement since the absorption mechanism is intact. Consequently, eight patients with a low serum B12 and a normal Schilling test, were treated with 100µg of oral cyanocobalamin, once a day for twelve months. Vitamin B12 levels were monitored before, during and after treatment. In this treatment group the median Vitamin B12 levels rose from 168 (147-177) to 415(252-621). In all eight patients (100%), the B12 level returned to normal after one year of oral replacement therapy.

Thirty-two (20%) of the 157 patients in whom serum ferritin levels were measured had developed a sub-normal level, comprising 17 females (24%) and 15 males (17%). Four (12%) of thirty-three patients with pre-op ferritin assays had subnormal levels compared to thirty one (21%) of one hundred and forty nine post-op patients.

Twenty-three (13.6%) of the 169 patients in whom serum folate levels were measured had developed a sub-normal level, comprising 10 females (14%) and 13 males (13%).

One (2%) of thirty-three patients with pre-op serum folate assays had sub-normal levels compared to twenty-two (21%) of one hundred and fifty five post-op patients. Thirty-three (21%) of the 158 patients in whom red cell folate levels were measured had developed a sub-normal level, comprising 18 females (26%) and 15 males (17%). One (3%) of forty patients with pre-op red cell folate assays had sub-normal levels compared to thirty-two (22%) of one hundred and forty four post-op patients

#### **4.4. Oral vitamin B12 replacement therapy**

Until recently, vitamin B12 replacement therapy was available only by intra-muscular injection of hydroxocobalamin. Cyanocobalamin is now available in tablet form allowing oral replacement. Those patients with vitamin B12 deficiency and a normal Schilling test were considered suitable for oral replacement since the absorption mechanism is intact. Consequently, eight patients with a low serum B12 and a normal Schilling test, were treated with 100µg of oral cyanocobalamin, once a day for twelve months. Vitamin B12 levels were monitored before, during and after treatment. In all eight patients the serum vitamin B12 levels returned to the normal range.

## 4.5 Discussion

These results form the largest reported study with the longest follow-up of haematological results after RP.

They suggest that at least one quarter of pouch patients will develop an abnormally low serum B12 after pouch surgery but this did not appear to be associated with a macrocytic anaemia. It is well established that humans have 3-4 years of stores of vitamin B12 if their supplies are completely cut off.<sup>245</sup> The steady decrease in B12 levels in 73% of patients with multiple B12 readings, suggests that over a longer follow-up period, a much higher percentage of patients will develop B12 deficiency. Since there was only one B12 deficient patient with proven bacterial overgrowth occurring in their ileal pouch, this does not appear to be cause of the deficiency. The normal Schilling test results in 94% of the patients with low B12 levels suggest that terminal ileal resection is also not the cause, but indicate that oral B12 replacement therapy could be successfully absorbed across the small bowel. As expected after normal Schilling tests, all study patients treated on a replacement regimen of 100µg of oral B12 (cyanocobalamin) once a day, had their B12 levels returned to normal after twelve months, and it would appear reasonable to recommend this treatment regimen in such cases. Other smaller studies with shorter follow-up found subnormal B12 levels in 0-10% of patients.<sup>60;61;94</sup> This was despite the fact that the percentage of abnormal Schilling tests was 5-75%.<sup>60</sup> No mention was made in these other studies, whether patients were receiving oral or parenteral B12 replacement therapy at the time of testing but one<sup>60</sup> described that, “obviously there were lots of patients on supplements at the time of the B12 test.” If B12 levels were checked within a few months of a Schilling test they would give a falsely high reading due to the large loading dose of B12 given during this test. M’Koma<sup>60</sup> calculated that a loop



ileostomy rendered inactive, the absorptive capacity of 45-212cm of small bowel, but found no correlation between the length of the excluded bowel and the occurrence of pathological values. Many studies show that a sufficient amount of B12 cannot be absorbed in the absence of the terminal ileum,<sup>69,246-251</sup> and one suggests that B12 deficiency further impairs B12 absorption<sup>252</sup>. We found no evidence of bacterial overgrowth in our B12-deficient patients though previous studies have reported this.<sup>253-255</sup> The abnormal growth of the bacterial flora, possibly associated with changes in the intrinsic factors, the ileal receptor for the intrinsic factor-B12 complex, bacteria utilising vitamin B12, and decreased transit time due to anatomic constraints, might interfere with vitamin B12 absorption<sup>256</sup>.

In conclusion, we found that at least one quarter of patients will have low serum B12 levels following RP and with longer follow-up this figure may increase significantly. This deficiency does not appear to be secondary to either; terminal ileal resection or bacterial overgrowth within the pouch. Patients should have their B12 levels monitored at regular intervals following pouch surgery and those with subnormal B12 levels should have them successfully restored to a normal value after a year's treatment with oral B12 replacement therapy.

In comparing the number of patients with an abnormally low haemoglobin, the study of M'Koma<sup>256</sup> had a higher percentage of anaemic patients prior to emergency surgery (67% compared to our 58%), and a half the percentage of anaemic patients prior to elective pouch formation (22% compared to 44%). It also reported only 4% of patients were anaemic one year after surgery compared to our 30%. At the same stage 23% of our patients had abnormally low serum ferritin levels. These abnormal results post-RP may be attributable to ; a nutritionally poor Glaswegian diet containing

insufficient iron, defective digestion, reduced absorption or continued blood loss-possibly due to “pouchitis” and/or “cuffitis”.

M’Koma’s results for serum folate reported 14% deficiency in emergency pre-op patients compared to our 25% but he found no abnormal folate levels post-RP in contrast to our findings of 21%. Red cell folate values are more informative since they reflect the folate levels over the previous three months and these were abnormally low in 22% of patients post-RP. Unlike vitamin B12, folate is absorbed in the proximal small bowel and the higher levels of deficiency found in this study may again be explained by poor diet or impaired intestinal absorption.

**CHAPTER FIVE**

**PROSPECTIVE AND  
RETROSPECTIVE BIOCHEMICAL  
AND NUTRITIONAL STUDIES  
FOLLOWING RESTORATIVE  
PROCTOCOLECTOMY**

## **5.1 Introduction**

### **5.1.1 Assessment of body composition**

To interpret weight change within patients is important to understand the distribution of weight between the various body compartments. For example, patients with advanced cancer that lose weight do so from the fat and muscle compartments and there is no loss in the non-muscle protein compartment.<sup>257;258</sup>

Components of the human body, in their most simple form can be divided into two compartments; fat and fat-free mass. A number of methods exist to measure these two compartments including: hydrostatic weighing, isotope dilution, skinfold anthropometry and bio-electrical impedance analysis (BIA). Hydrostatic weighing is a technique which uses the Archimedes principle to estimate fat and fat free mass<sup>259;260</sup> and involves the complete submersion of the patient in water. Reproducibility of this technique is reported as showing variation of 1.2%.<sup>67</sup> Such methodology can prove impractical and it was considered unsuitable for the assessment of body mass of our patient group.

Isotope dilution to measure body composition<sup>261;262</sup> involves the injection of a known concentration of radioactively labelled solution into the body. After equilibrium has been reached, blood samples are taken to establish the degree of dilution and hence estimate the total body water. Clearly, this is an invasive procedure involving venesection and exposure to radiation and hence was considered unsuitable for regular body composition assessment in our study group.

Skin anthropometry and weight measurements are far more practical, non-invasive methods of nutritional assessment so these were employed.

Total body weight is equivalent to the sum of the fat and the fat-free mass.<sup>259</sup> Most human fat is stored subcutaneously, consequently a variety of relatively painless

skinfold thickness measurements have been developed to estimate total body fat. On the negative side, this technique is relatively imprecise and its variation is reported as 6-24%.<sup>263</sup> Error minimisation can be achieved by ensuring measurements are taken by the same observer and are taken in triplicate, recording the mean score.<sup>264</sup>

BIA has been used to estimate both intra- and extra-cellular body water and subsequently, as an index of total body water. Hoffer et al.<sup>265</sup> demonstrated a correlation of  $r=0.92$  between body electrical impedance and total body water. The principle behind this method relates to the conduction of an applied electrical current through the body, since the fat free tissues, which consist of both intra- and extra-cellular, will conduct the current, whilst the fat acts as an insulator. When an alternating current is applied to the body, the impedance to the current is its frequency. At low frequencies( $\approx 1\text{kHz}$ ) the current passes mainly through extra-cellular fluid, whilst at higher frequencies( 500-800kHz) it passes through both the extra- and intra-cellular fluids.<sup>266;267</sup> This bio-electrical impedance measurement enables the calculation of total body water which is then extrapolated to determine the fat- free mass.

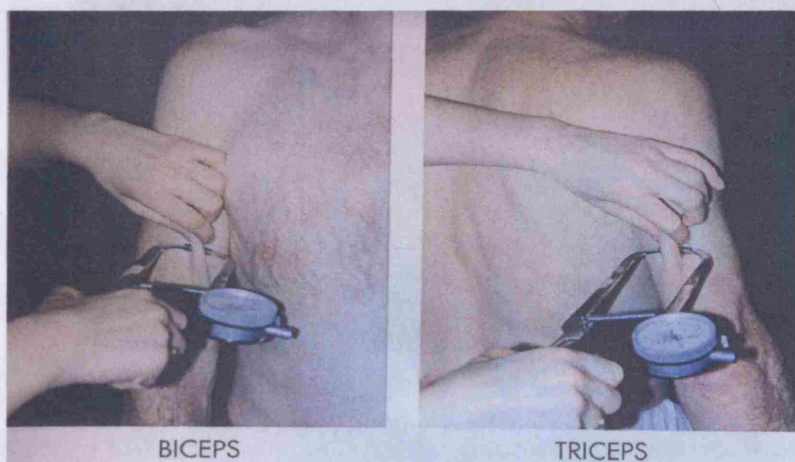
Commercial multi-frequency bio-electrical impedance analysers have been developed which provide a simple, portable and non-invasive estimation of body composition. Inevitably BIA will involve a degree of error. Hannan et al.<sup>266;268</sup> reported errors of approximately four litres in the estimation of total body water. One study compared the use of BIA with isotope dilution to predict total body water in groups of cancer patients and healthy volunteers. They reported that total body water was consistently over-estimated in the underweight patients when predictive formulae were used which had been developed in normal volunteers. Royall et al.<sup>269</sup> reported over-estimation of total body water using BIA in a group of malnourished patients with Crohn's disease.

It should be possible to characterise post-operative weight change as an alteration in either; adipose tissue, protein mass or intra/extra-cellular fluid. Despite the intrinsic errors associated with skinfold anthropometry, BIA and weight, these methods were employed to assess alterations in the nutritional status and body composition of the patients. They were selected because they are; simple, portable, repeatable and non-invasive.

### 5.1.2 Anthropometry

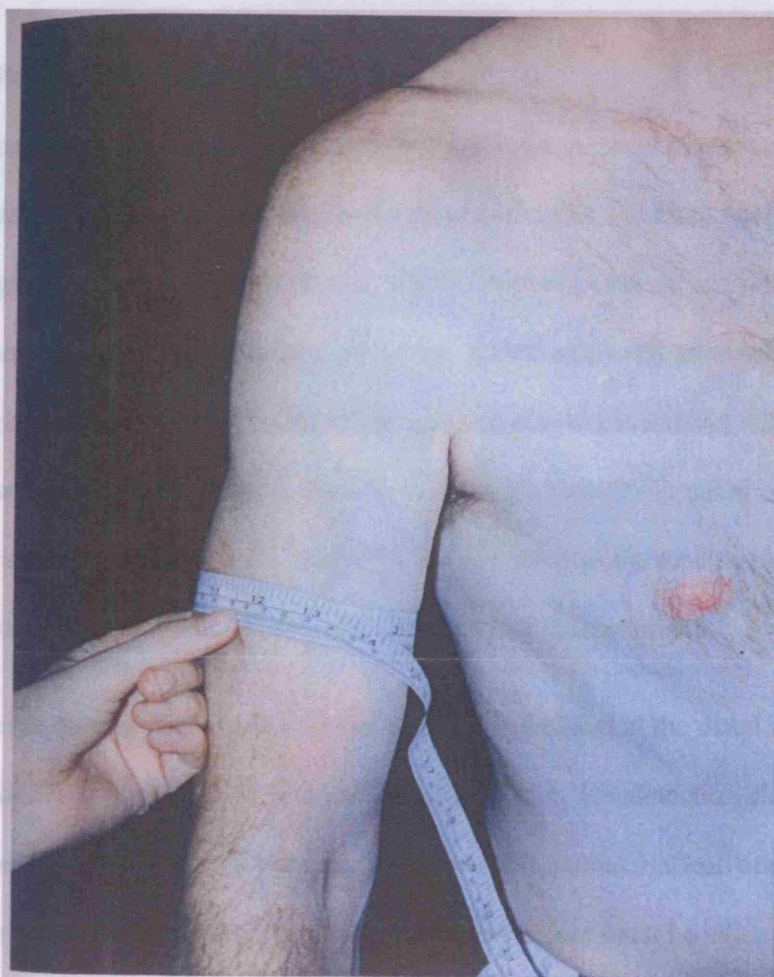
In the present studies, anthropometry was undertaken using Harpenden skinfold callipers (British Indicator Ltd. W. Sussex, UK) They produce a constant spring pressure of  $10\text{g/mm}^2$  throughout its entire range, which is recommended for the accurate measurement of skinfold thickness. A stretch-resistant measuring tape was used to accurately measure limb circumference and to locate anthropometry measurement sites. Various sites have been used to estimate body fat using numerous equations.<sup>259;270-273</sup> The measurements undertaken to estimate body composition in the present studies are detailed below. All measurements were undertaken on the right side of the body and the patient was standing with the arm relaxed at their side.

**Figure 33** Biceps and triceps skinfold thickness measurement with calipers



The biceps skinfold thickness was measured on the anterior surface of the biceps midway between the axillary fold and the antecubital fossa. The triceps site was measured directly over the triceps muscle on the posterior midline of the upper arm, at the midpoint between the acromial process of the scapula and the olecranon process of the ulna. All skinfold measurements were taken three times until the readings corresponded within one millimetre and the average score was then recorded to the nearest 0.5 millimetre.

**Figure 34** Mid-upper arm circumference measurement



The mid-upper arm circumference was taken at the midpoint between the olecranon and the acromial processes. The tape was maintained in a horizontal position touching the skin but not compressing it. Each individual's height was measured, to the nearest centimetre, using stadiometer with the patient standing in stocking feet. The weight, to the nearest kilogram, was obtained using beam-balance scales (model 424, Weylux, Cambridge, UK). Weight is routinely used as an indicator of over- or under-nutrition, however the relationship of height to weight enables useful analysis of the data. The following equation was used to calculate body mass index:

$$\text{Body Mass Index (BMI)} = \text{Weight(Kg)} \div \text{Height(m)}^2$$

### **5.1.3 Bio-electrical impedance spectrum analysis**

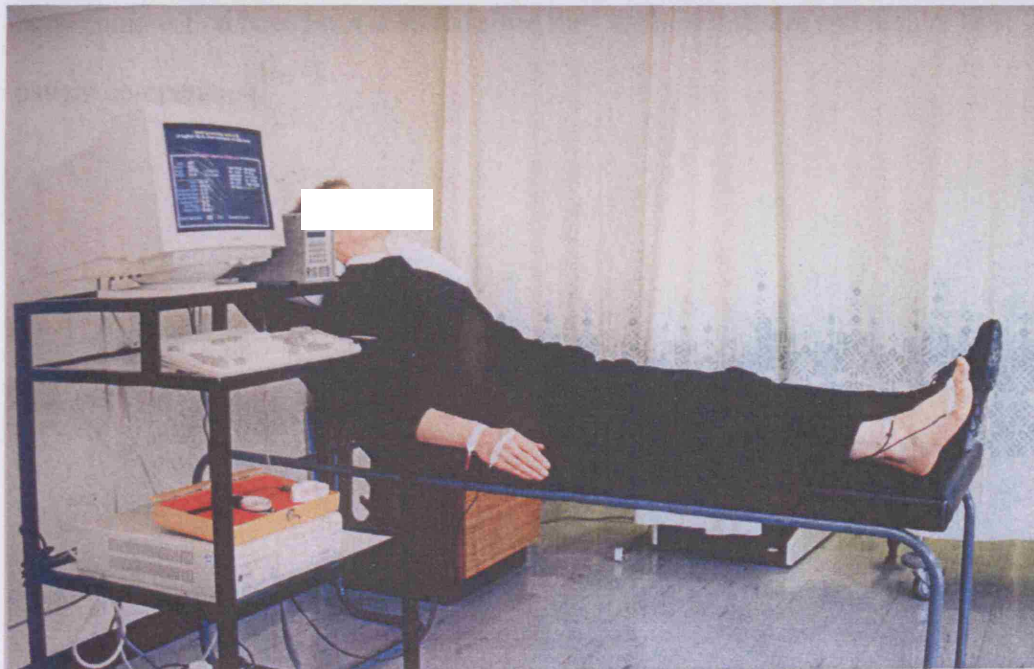
In the present studies, multi frequency bio-impedance measurements were undertaken using a Xitron 4000B complex bio-impedance spectrum analyser (Xitron technologies inc. San Diego, Ca. 92121, USA). The patients were positioned on a clinical couch in the supine position with legs slightly apart and arms relaxed at the side but not touching the body. (**fig 35**) The patient was not in contact with any metal surfaces and the leads from the analyser were not in contact with metal or with each other. Since electrode placement can influence accuracy of the results, physical landmarks were also used to reduce the variability of measurements.

The current source electrodes were placed on the right hand at the distal metacarpal joint, and on the right foot at the distal metatarsal joint. The detection electrodes were also placed on the right limbs between the lateral and medial malleoli and between the distal prominence of the radius and the ulna (**fig.36**). Care must be taken when using this analyser to obtain accurate measurements. The electrode placement is important since the 4000B analyser will accurately measure parameters between the points of



contact. Consequently any variation in the placement of the electrodes may cause an apparent reduction in the repeatability of measurements. The electrodes recommended by the manufacturer of the bio-impedance analyser were used. These are disposable, self-adhesive, pre-gelled electrodes with a surface contact area of  $14.5\text{cm}^2$ . The skin was wiped with 70% isopropyl alcohol impregnated swabs prior to placement of the electrodes. Before each patient measurement, the analyser was calibrated using a test resistor( $422\Omega$ ) supplied by the manufacturer. Resistance and reactance were measured using 50 frequencies between 5kHz and 1 MHz.

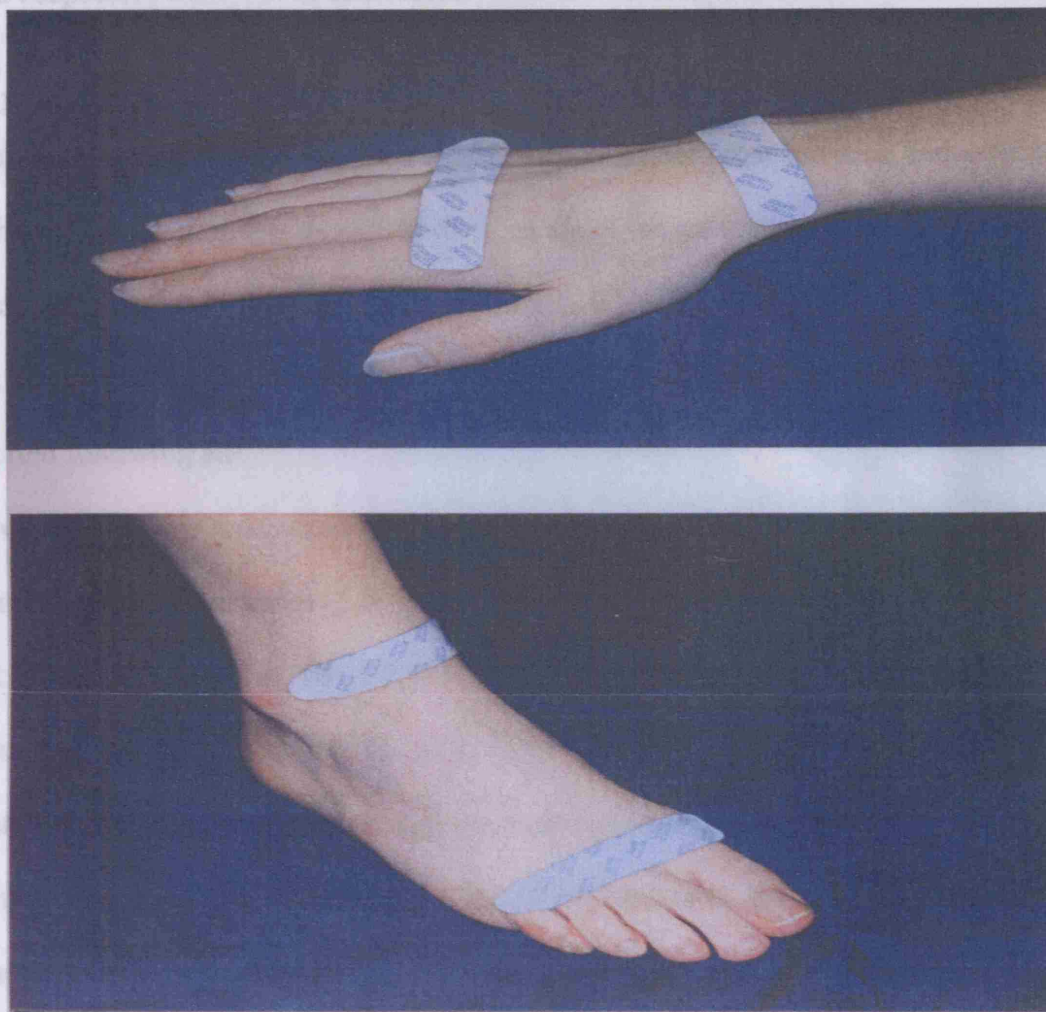
**Figure 35** Equipment set-up for bio-electrical impedance spectrum analysis



A modelling program, supplied by the manufacturer, was used to carry out cole-cole analysis of the recorded spectrum and quantitative statistics were used to establish the quality of the fit of the data to this model. This analysis predicts the impedance at frequencies zero and infinity, which corresponds to extracellular resistance and total

body water resistance respectively. These are then combined with the patient's weight and height to calculate the extracellular, intracellular and total body water volumes. In summary, it is important to measure changes in body composition to assess alteration in nutritional status. In the following studies, the fat and fat-free mass model of body composition will be applied. These compartments will be assessed using skinfold anthropometry and bio-electrical impedance as described in this chapter. To limit observer variability using skinfold anthropometry, all assessments will be undertaken by one observer and the average of three measurements recorded. To reduce the error involved in bio-electrical impedance, electrodes will be positioned using physical landmarks and repeated measurements taken. These techniques for assessment of body composition have been selected since they are non-invasive and require little patient co-operation.

**Figure 36** Correct distal limb placement of bio-electrical impedance electrodes



## **5.2 Prospective nutritional assessment before and after restorative proctocolectomy**

### **5.2.1 Introduction**

Restorative proctocolectomy, involving resection of the large intestine and a portion of the terminal ileum, has provoked concern about the subsequent nutritional status of patients undergoing this procedure. Vitamin B12 deficiency has been described following pouch formation.<sup>60</sup> More recently, changes in the lipid profile have been reported following RP.<sup>60</sup>

However, it is now recognised that plasma concentrations of many vitamins and trace elements and their associated binding proteins and lipid fractions, are decreased as part of the presence of the systemic inflammatory response and therefore such circulating concentrations may not reflect tissue levels.<sup>32;122;124</sup>

Therefore, it is not clear whether such biochemical changes are specific to this clinical scenario or part of a more general nutritional disturbance, driven by inflammation in these patients.

The aim of the present study was to examine the relationship between body composition, nutritional status and the systemic inflammatory response prior to colectomy and pre- and post ileo-anal pouch formation in patients with UC.

### 5.2.2 Patients and study design

All patients within this study had undergone RP for UC. Venous blood samples (serum/ lithium heparin/ EDTA) were withdrawn for analysis of a panel of analytes forming a “nutritional screen” at several time points;

prior to restorative proctocolectomy,  
prior to closure of defunctioning loop ileostomy,  
3 months post-ileostomy closure  
6 months post-ileostomy closure and  
12 months post-ileostomy closure.

### 5.2.3 Methods

Height and weight were measured, and body mass index (BMI) was calculated using the formula  $BMI (kg/m^2) = \text{weight (kg)} / \text{height (m}^2\text{)}$ . Biceps and triceps skin-fold thickness were measured with Harpenden skin-fold calipers (British Indicator Ltd., West Sussex, United Kingdom) using established techniques. Mid-upper arm circumference were measured using a stretch-resistant measuring tape. Total body water was measured as previously described,<sup>1</sup> using a bioelectrical impedance system (Xitron 4000B; Xitron Technologies, San Diego, CA, USA). The error of the method is approximately 10%.

Calcium, phosphate, total protein, albumin and C-reactive protein concentrations and bilirubin, alkaline phosphatase, aspartate transaminase, alanine transaminase activity were assayed on an Olympus AU5200 analyser (Olympus Optical Ltd., Tokyo, Japan) using standard methods. Intra- and inter-assay coefficients of variation were better than 5% and 10% respectively. The calcium concentrations were adjusted for albumin, the “normal” albumin being considered to be 44 i.e. Adjusted calcium = measured calcium + ((44- measured albumin) x 0.02)

IgM, IgG and IgA levels were determined by nephelometry<sup>125</sup>

Vitamins A, B1, B2, B6, B12, C and E were measured using high-pressure liquid chromatography as previously described.<sup>274</sup>

Trace elements magnesium, zinc and copper were measured by using an inductively coupled plasma atomic emission spectrometer (Atomspek 16, Thermal Jarell Ash, Cambridge, UK). The imprecision of this method was less than 3% within each batch.

Selenium was determined by graphite furnace atomic absorption spectrophotometry (Perkin-Elmer 1100B atomic absorption spectrometer with HGA 700 graphite furnace, Perkin-Elmer, Cambridge, UK) by using palladium as a matrix modifier.

The imprecision of this method was less than 3% within each batch. Glutathione peroxidase activity was measured as an additional assessment of selenium status.

Haemoglobin was measured by a Coulter GenS automated FBC analyser. Ferritin, red cell and serum folate was measured using a microparticle enzyme immunoassay method (Abbott Diagnostics).

#### **5.2.4 Statistics**

Data are presented as median and range. Data from different time periods were tested for statistical significance using the Friedman test and where appropriate comparisons of data from different time periods were carried out using the Wilcoxon signed rank test. Analysis was performed using SPSS software (SPSS Inc., Chicago, Illinois, U.S.A.).

### 5.2.5 Results

Thirteen patients who underwent RP were entered into the prospective study. Two of these patients experienced failure of their pouch prior to ileostomy closure, requiring pouch excision and formation of a permanent ileostomy. Following ileostomy reversal, two other patients had a complicated clinical course with pelvic sepsis at 3 months and therefore were excluded from analysis.

The normal range for measured parameters and the characteristics of the remaining nine patients pre-RP and pre-ileostomy closure are shown in **table 12**. The median time to ileostomy closure was 3.1 months (range 1.9-8.9months). Prior to RP almost all measured parameters were within the normal range. However, circulating concentrations of zinc were at the bottom end of the normal range.

Between RP and ileostomy closure there were significant reductions in weight ( $p<0.05$ ), biceps and triceps skinfold thicknesses ( $p<0.05$ ), mid-upper arm circumference ( $p<0.05$ ) and total body water ( $p<0.05$ ). There was a fall in total protein ( $p<0.05$ ), IgA ( $p<0.01$ ), haemoglobin concentrations ( $p<0.05$ ) and in both plasma and red cell glutathione peroxidase ( $p<0.05$ ). There was also a trend towards a fall in vitamins B2 ( $p<0.10$ ) and B12 ( $p<0.10$ ) and zinc concentrations. In contrast, there was a significant increase in serum phosphate ( $p<0.05$ ) and alkaline phosphatase activity ( $p<0.01$ ).

The measured parameters of patients 3-12 months following ileostomy closure are shown in **table 13**. There were no significant changes in almost all measured parameters over this period. However, there was an increase in both plasma and red cell glutathione peroxidase ( $p\leq 0.05$ ) and a trend towards an increase in calcium ( $p<0.10$ ), magnesium ( $p<0.10$ ) and MCV ( $p<0.10$ ) over the follow-up period. In

contrast there was a fall in total protein ( $p<0.05$ ) and copper ( $p<0.05$ ) and a trend towards a fall in vitamin A ( $p<0.10$ ).

**Table 12.** Characteristics and biochemical profile of patients prior to undergoing restorative proctocolectomy (RP) and ileostomy closure (IC).

	Reference range	Pre- RP (n=9)	Pre-IC (n= 9)	p-value
Age (yrs)		52 (29-63)		
Sex (m/f)		6/ 3		
Weight (kg)		63.5 (53.2-89.0)	62.1 (50.2-82.0)	0.011
Biceps skinfold thickness (mm)		4.2 (2.4-7.7)	3.4 (1.9-4.6)	0.011
Triceps skinfold thickness (mm)		6.4 (3.4-10.2)	5.6 (3.0-9.0)	0.011
Mid-upper arm circumference (cm)		28.0 (26.0-31.0)	26.5 (25.0-29.0)	0.014
Total body water (l)		36.1 (24.7-46.1)	32.9 (22.5-43.3)	0.011
BMI	20-25	25.1 (19.0-26.1)	22.3 (17.7-24.3)	0.011
Calcium (mmol/l)	2.2-2.6	2.45 (2.25-2.45)	2.40 (2.25-2.45)	0.739
Phosphate (mmol/l)	0.7-1.4	1.20 (0.80-1.45)	1.25 (1.10-1.70)	0.017
Total protein (g/l)	62-82	81 (71-85)	78 (67-83)	0.048
Albumin (g/l)	35-55	47 (43-81)	45 (42-72)	0.136
C-reactive protein (mg/l)	<6	<6 (<6-20)	<6 (<6-10)	0.285
IgM (g/l)	0.4-3.0	1.55 (1.20-2.30)	1.60 (1.30-2.00)	0.942
IgG (g/l)	6.0-15.0	13.8 (9.6-14.6)	14.2 (8.9-15.6)	0.138
IgA (g/l)	0.8-4.5	2.80 (1.40-3.80)	2.50 (1.20-3.50)	0.007
Bilirubin (umol/l)	3-22	9.0 (7.0-16.0)	12.0 (7.0-16.0)	0.141
Alkaline phosphatase (U/l)	80-280	175 (130-540)	210 (155-840)	0.008
Aspartate trasaminase (U/l)	12-48	23 (9-99)	26 (19-86)	0.137
Alanine transaminase (U/l)	3-55	21 (9-123)	28 (15-136)	0.678
Glutathione peroxidase Plasma (U/l)	200-1000	630 (337-988)	574 (210-927)	0.021
Red cell (U/gHb)	20-70	39 (19-57)	32 (23-48)	0.049
Magnesium (mmol/l)	0.70-1.00	0.80 (0.71-0.90)	0.80 (0.76-0.90)	0.944
Zinc (umol/l)	12-18	12.0 (9.0-14.5)	10.8 (9.0-13.0)	0.075
Copper (umol/l)	10-22	19.5 (10.5-32.0)	16.5 (15.0-30.0)	0.259
Selenium (umol/l)	0.80-2.00	1.01 (0.49-1.30)	1.05 (0.38-1.27)	0.235
Vitamin A (umol/l)	1.0-2.0	1.9 (1.0-3.4)	1.9 (1.4-4.1)	0.888
Vitamin B1 (ng/ gHb)	275-675	390 (290-570)	420 (300-690)	0.213
Vitamin B2 (% activation)	<70	30 (20-50)	25 (5-45)	0.084
Vitamin B6 (nmol/l)	17-135	68 (30-89)	59 (22-83)	0.108
Vitamin B12		270 (180-702)	249 (111-543)	0.051
Vitamin C (umol/l)	11-114	43 (10-91)	51 (10-152)	0.325
Vitamin E (umol/l)	14-39	23 (17-38)	25 (20-35)	0.232
Haemoglobin (g/dl)		14.4 (11.2-17.1)	12.6 (11.0-14.8)	0.015
Ferritin		13.9 (7.3-41.8)	21.4 (6.5-49.6)	0.859
Red cell folate		325 (220-747)	347 (236-555)	0.953
Serum folate		8.6 (3.1-14.1)	11.0 (5.4-14.9)	0.407
MCV		88.7 (74.3-92.8)	87.2 (82.6-90.3)	0.483



**Table 13** Longitudinal biochemical profile of patients with a normal functioning pouch.

	3 mths (n= 9)	6 mths (n= 9)	12 mths (n= 9)	ANOVA p-value
Weight (kg)	60.9 (53.0-81.0)	59.8 (53.2-79.0)	60.1 (50.7-83.0)	0.819
Biceps skinfold thickness (mm)	3.6 (3.0-4.4)	3.4 (2.1-5.2)	3.2 (2.2-5.0)	0.972
Triceps skinfold thickness (mm)	6.0 (4.2-9.4)	6.4 (3.7-9.8)	6.2 (3.4-9.9)	0.819
Mid-upper arm circumference (cm)	26.5 (25.5-29.0)	26.5 (25.5-29.0)	26.5 (24.5-30.0)	0.814
Total body water (l)	35.5 (22.8-44.0)	35.7 (24.9-44.5)	36.0 (25.1-43.0)	0.121
BMI	20.9 (18.6-23.7)	21.0 (17.7-25.0)	20.8 (18.1-25.6)	0.819
Calcium (mmol/l)	2.35 (2.20-2.45)	2.35 (2.30-2.40)	2.45 (2.25-2.60)	0.089
Phosphate (mmol/l)	1.35 (1.00-1.80)	1.25 (0.90-1.40)	1.35 (1.00-1.50)	0.412
Total protein (g/l)	78 (72-85)	75 (67-80)	75 (62-82)	0.035
Albumin (g/l)	47 (42-72)	47 (39-73)	45 (37-69)	0.648
C-reactive protein (mg/l)	<6 (<6-10)	<6 (<6-8)	<6 (<6-12)	0.497
IgM	1.6 (1.3-2.3)	1.5 (1.2-2.2)	1.55 (1.1-2.2)	0.409
IgG	13.9 (10.6-17.2)	12.9 (9.8-16.5)	12.9 (9.0-16.2)	0.177
IgA	2.7 (1.3-3.9)	2.9 (1.3-4.1)	2.6 (1.2-4.5)	0.970
Bilirubin (U/l)	9 (7-17)	12 (3-23)	12 (9-22)	0.117
Alkaline phosphatase (U/l)	210(135-1090)	190 (140-1120)	180 (135-1060)	0.773
Aspartate transaminase (U/l)	24 (12-78)	21 (16-71)	26 (13-156)	0.175
Alanine transaminase	23 (8-139)	22 (11-91)	19 (13-28)	0.337
Glutathione peroxidase	610 (270-967)	580 (382-826)	653 (415-1021)	
Plasma				0.050
Red cell	30 (22-56)	29 (24-52)	41 (19-62)	0.021
Magnesium (mmol/l)	0.82 (0.75-0.90)	0.84 (0.70-0.90)	0.85 (0.75-1.0)	0.082
Zinc (umol/l)	12.5 (7.5-22.0)	12.0 (8.5-15.5)	12.0 (10.0-19.0)	0.607
Copper (umol/l)	18.5 (16.5-40.0)	17.0 (14.5-29.0)	17.0 (13.5-29.5)	0.037
Selenium	0.93 (0.38-1.25)	0.88 (0.60-1.40)	1.06 (0.58-1.23)	0.462
Vitamin A	1.9 (1.4-3.0)	1.9 (1.3-3.8)	1.7 (1.0-2.9)	0.072
Vitamin C	46 (10-68)	62 (10-131)	54 (10-94)	0.435
Vitamin E	27 (15-39)	25 (15-49)	24 (20-66)	0.968
Vitamin B1	420 (350-620)	420 (320-730)	410(240-654)	0.549
Vitamin B2	20 (5-40)	10 (5-30)	15 (5-40)	0.531
Vitamin B6	33 (16-77)	51 (12-102)	48 (27-155)	0.462
Vitamin B12	227 (122-603)	320 (118-439)	254 (107-450)	0.121
Haemoglobin (g/dl)	12.8 (10.3-15.8)	13.4 (11.1-15.2)	14.0 (10.6-15.3)	0.717
Ferritin	9.5 (4.9-50.1)	28.0 (2.4-59.8)	13.5 (3.8-61.9)	0.097
Red cell folate	328 (144-765)	269 (257-450)	310 (179-463)	0.276
Serum folate	8.3 (4.2-14.2)	9.5 (6.4-14.3)	9.5 (6.6-16.9)	0.121
MCV	86.4 (76.8-91.5)	84.7 (80.8-91.5)	88.1 (79.1-92.4)	0.062

### 5.2.6 Discussion

The results of the present study show that, prior to uncomplicated RP, the majority of patients have normal body composition and nutritional profile including vitamins and trace elements. Furthermore, although the period between RP and ileostomy closure was associated with significant weight loss there were minimal changes in the nutritional screen with the exception of the fall in glutathione peroxidase activity. Lastly, there was a stabilisation in the body composition and nutritional profile and a recovery in glutathione peroxidase activity over 12 months follow-up of patients with normal functioning pouch.

An interesting feature of the present study was that systemic inflammatory response, as evidenced by elevated circulating concentrations of C-reactive protein, was not significantly activated at any of the assessment times of the study, not even at the time of weight loss during the period between restorative proctocolectomy and ileostomy closure. This is somewhat surprising, given the well-documented relationship between activation of the systemic inflammatory response and weight loss in other disease states.<sup>218;275</sup> This would suggest that the weight loss during this time was probably due to decreased energy intake.

There have been few longitudinal studies which have examined the nutritional status of patients undergoing restorative proctocolectomy. M'Koma et al.<sup>39</sup> reported that in 83 patients, pre-operatively, there was a significant proportion of patients with low calcium and magnesium concentrations which subsequently recovered over the period of 3 years following ileostomy closure. Similar findings were reported with respect to haemoglobin and vitamin B12. The magnitude of the systemic inflammatory response during the study was not documented.

Kuisma et al.<sup>34</sup> examined the effect of restorative proctocolectomy (n=104) on fat-soluble vitamins and vitamin B12 levels. Vitamin deficiency was less than 10% in this cohort and they noted that this tended to be in those patients inflammation in the proximal limb. Similarly, Tiainen et al. reported that anaemia was a significant factor in those patients who developed pouchitis.<sup>98</sup>

The majority of patients had biochemical parameters in the normal range prior to surgery. The magnitude of the systemic inflammatory response during the study was not documented.

In summary, uncomplicated restorative proctocolectomy appears to be associated with minimal nutritional derangements in the short term after closure of ileostomy. The period prior to closure appears to be associated with weight loss but this does not appear to impact significantly on other blood or nutritional parameters.

### **5.3 Long-term biochemical and nutritional outcome following RP**

#### **5.3.1 Introduction**

RP alters the normal gastrointestinal physiology. There is loss of the absorptive capacity and bacterial flora of the large bowel. The pouch, created to reduce bowel frequency, leads to an abnormal bacterial flora in the ileum and prolonged small bowel transit.<sup>53;94;257</sup> This may in turn, explain why pouch patients develop bile salt malabsorption.<sup>89;276;277</sup> Despite the fact that many thousands of pouch operations have been performed worldwide, little is known about the long term nutritional sequelae of RP.. Earlier in this chapter we have described, in a small prospective study of nutritional status over 12 months following RP, that significant changes are few and modest in their depletion. We also found that few patients had evidence of a systemic inflammatory response during this period and the presence of a systemic inflammatory response was unlikely to be an important factor in the nutritional status of these patients.

Vitamins and trace elements are important, although incompletely understood, components of human nutrition, that are derived from the diet and believed to be absorbed in the small intestine.<sup>278</sup> Some, such as copper, zinc and manganese are excreted in bile but may be reabsorbed by the small intestine via the enterohepatic circulation. Others (eg selenium) are excreted in the urine. It is known that either deficiency or toxicity of an individual vitamin or trace element may produce severe symptoms and ill-health.<sup>205</sup>

As stated earlier in chapter 4, many micronutrients such as B-vitamins have large tissue stores and that depletion even in absence of absorption from the diet may take several

years for significant depletion to occur. Therefore, the aim of the present study was to examine micronutrient concentrations 2-5 years and 5-10 years following RP.

### **5.3.2 Methods**

Patients who had undergone RP more than 2 years previously were studied. Venous blood samples (serum/ lithium heparin/ EDTA) were withdrawn for the measurement of a panel of analytes forming a “nutritional screen” at a single time point. However, body composition measurements were not carried out at this time, since data from previous time periods was not available and due to pressure on clinic time.

The same methodology, as employed in section 5.2.3 was used.

Patients were excluded if they were taking oral nutrient supplements at the time of the nutrition screen.

### **5.3.3 Statistical analysis**

Data are presented as median and range. Data from different time periods were tested for statistical significance using the Mann-Whitney U-test. Analysis was performed using SPSS software (SPSS Inc., Chicago, Illinois, U.S.A.).

### **5.3.4 Results**

A total of 113 patients were entered into the cross sectional study. These were grouped according to whether they were 2-5 years (n=65) or 5-10 years (n=48) post-RP.

The normal range for measured parameters and the micronutrients profiles of the two time periods are shown in **table 14**.

At 2-5 years following restorative proctocolectomy and ileostomy closure the median values for all analytes were within the normal reference range, Only a few values were below this range including C-reactive protein. The results were similar at the 5-10 year period and there were no significant differences in any of the parameters when corrected for multiple comparisons.

**Table 14** Characteristics and micronutrient profile of patients following restorative proctocolectomy (RP) and ileostomy closure (IC).

	Reference range	Median (range)		p-value
		2-5 years (n= 65)	5-10 years (n= 48)	
Age (yrs)		34 (16-57)	35 (16-59)	0.678
Sex (m/f)		34/ 31	28/ 20	0.526
Adjusted Calcium (mmol/l)	2.2-2.6	2.35 (2.20-2.55)	2.35 (2.15-2.50)	0.311
Phosphate (mmol/l)	0.7-1.4	1.08 (0.63-1.45)	1.10 (0.75-1.60)	0.260
Total protein (g/l)	62-82	74 (63-91)	74 (62-86)	0.512
Albumin (g/l)	35-55	44 (38-52)	44 (33-51)	0.512
C-reactive protein (mg/L)	<10	<6 (<6-44)	<6 (<6-192)	0.366
Vitamin A (umol/l)	1.0-2.0	1.8 (0.7-2.6)	2.0 (0.9-2.9)	0.167
Vitamin B1 (ng/ gHb)	275-675	413 (250-650)	418 (250-510)	0.717
Vitamin B2 (% activation)	<70	18 (<5-50)	20 (<5-55)	0.293
Vitamin B6 PA (nmol/l)	10-55	18 (9-47)	19 (6-75)	0.815
Vitamin B6 PLP	18-135	28 (10-59)	24 (10-54)	0.538
Vitamin C (umol/l)	11-114	35 (10-115)	31 (10-103)	0.334
Vitamin E (umol/l)	14-39	27 (11-56)	27 (12-53)	0.923
Magnesium (mmol/l)	0.70-1.00	0.78 (0.63-0.97)	0.81 (0.70-0.92)	0.053
Zinc (umol/l)	12-18	12 (7-20)	13 (2-20)	0.764
Copper (umol/l)	10-22	19 (9-30)	19 (9-34)	0.985
Selenium (umol/l)	0.80-2.00	0.92 (0.45-1.32)	0.89 (0.44-1.13)	0.216
Glutathione peroxidase				
Plasma (U/l)	200-1000	628 (280-949)	614 (349-859)	0.355
Red cell (U/gHb)	20-70	38 (19-67)	35 (16-56)	0.288

## 5.6 Discussion

The results of the present study show that on long term follow-up (2-5 and 5-10 years) of patients who have undergone RP, the majority of patients maintain a normal micronutrient profile.

Given the loss of a large amount of intestinal absorptive surface area associated with this procedure it is perhaps surprising that few micronutrient deficiencies were seen in the present study. However, from our previous study in this chapter, it is clear that a functioning pouch is associated with weight stabilisation and therefore adequate energy intake. The results of the present long term study suggest that micronutrient intake is also adequate in these patients.

An interesting feature of the present study was that systemic inflammatory response, as evidenced by elevated circulating concentrations of C-reactive protein, was not significantly activated at any of the assessment times of the study. The lack of a systemic inflammatory response is also likely to contribute to the maintenance of a “normal” micronutrient profile.

There have been few long term studies which have examined the nutritional status of patients who had undergone RP. The results of the present study are consistent with reports that micronutrient concentrations may recover following successful surgery.<sup>279</sup> Also, more recent studies that suggest that vitamin deficiency is a relatively rare event (<10%) in these patients, even in those followed-up for 4-6 years.<sup>276</sup>

In summary, uncomplicated RP appears to be associated with minimal nutritional derangements in the long term after closure of ileostomy.



**CHAPTER SIX**

**CONCLUSIONS  
AND FUTURE WORK**

## **6.1 Pre-dysplasia, dysplasia and carcinoma in the rectal “cuff”**

It is well recognised that patients with chronic UC have an increased risk of developing colorectal carcinoma when compared with control subjects <sup>164;165 166</sup> and the presence of colonic dysplasia has been shown to be a risk factor which warrants careful follow up. <sup>167;168</sup> This risk also applies to patients with UC who have undergone surgery where rectal mucosa is preserved, including RP patients who retain a rectal “cuff”.

Earlier studies documented a 6% risk of developing a rectal cancer during the subsequent 10-20 years after colectomy and ileo-rectal anastomosis (IRA), <sup>169-174</sup> rising to 15-20% after 30 years. <sup>169;175</sup> It was noted that the cancer risk was reduced if there was no dysplasia or carcinoma in the resected specimen.

According to these calculations, since RP retains a 1-3 cm cuff of columnar epithelium, the hypothetical risk for these pouch patients, extrapolated from the available data for patients with UC who have had a IRA, has been calculated to be 1% at 30 years. <sup>36</sup> This would double to 2% if an additional 2 cm of rectal mucosa was inadvertently retained above the anal canal.

Despite these low theoretical risk rates for malignant change in the rectal cuff, in the absence of established figures for long-term follow-up post-RP, it has been considered necessary to perform follow-up cuff surveillance biopsies.

It is important to document that the procedure of performing surveillance biopsies on patients with UC failed to reveal any neoplastic transformation in 4 out of 6 patients who subsequently developed adenocarcinoma cancer in Felipe et al.’s study.

In the present study there was no evidence of either dysplasia or carcinoma in the rectal cuff at up to 12 years post pouch formation and up to 32 years post diagnosis of UC. These data from patients are similar to those predicted by the theoretical analysis

above.

Eight published series from six centres, have reported the risk of dysplasia and carcinoma in cuff biopsies.<sup>36;178;180;228;280</sup> Although the follow up is short in most of these studies, taken together, only four patients had persistent evidence of dysplasia in the cuff. In two cases this was low-grade dysplasia.<sup>178</sup> Importantly, one of these patients had high grade dysplasia and the other, low-grade dysplasia, in the original colectomy specimen. O' Riordain et al.<sup>177</sup> reported two patients with persistent evidence of high grade dysplasia in the cuff. Both patients had either high grade dysplasia or carcinoma in the original colectomy specimen.

This observation is clinically significant because although the overall dysplasia risk in the cuff is small, it was high for those patients who had evidence of either high grade dysplasia or carcinoma in the original resection specimen. One of these studies estimated the risk of developing cuff dysplasia at 10% if there was dysplasia in the colectomy specimen, and 25% if there was carcinoma in the original specimen.<sup>177</sup>

None of the patients in the present study had either high grade dysplasia or carcinoma in the original resection specimens which may explain why we have not found any dysplasia during follow-up.

Thompson-Fawcett et al.(DCR'00) remarked that although the presence of neoplasia in the colectomy specimen seems to indicate a higher risk of neoplasia in the columnar cuff, the absence of neoplasia does not necessarily predict a low risk for the columnar cuff. For patients who have a colectomy within ten years of the onset of UC, there is not time for the mucosa to express its malignant potential. Some patients who have had a RP and retained columnar cuff would have developed neoplasia in their colons if they were able to keep them long enough, and these cases may be at risk of columnar cuff neoplasia. Furthermore, in long standing disease if the

proctocolectomy specimen is not thoroughly sampled by the pathologist, foci of dysplasia may be missed, giving false reassurance.

Overall, the conclusion drawn from this study is that, since the incidence of cuff dysplasia is so low, it will require further longer-term follow up studies to truly determine the risk of cuff dysplasia following RP and to indicate how frequently cuff surveillance biopsies should be performed. Such information should prove valuable in terms of preservation of resources. It remains uncertain whether advice concerning a delay in the commencement of cuff-surveillance biopsies should be relative to the number of years after RP or number of years after diagnosis of UC.

Overall we recommend that those patients with no evidence of dysplasia or carcinoma in the resected large bowel probably do not need surveillance biopsies of the rectal cuff in the first 5-10 years after surgery. This will save both, resources and reduce the discomfort/ inconvenience for patients . Further long-term studies are required to prove beyond doubt that it is safe to only commence cuff surveillance after a specified time period after diagnosis or RP. Regular surveillance biopsies should continue to be offered to patients who have undergone RP for UC until further information is available.

This protocol should not be applied to patients who have either high-grade dysplasia or carcinoma in the resected large bowel since there is evidence that these patients have a high risk of developing dysplasia in the cuff .

If pre-operative evidence exists that dysplasia or carcinoma is present in the colon prior to surgery, then a stapled RP should probably be avoided and a hand-sewn anastomosis performed along with mucosectomy.

In the event that a patient who has had a stapled RP develops dysplasia in the cuff, it is unclear how this should be managed . In particular it is unknown whether low grade

dysplasia invariably progresses to high grade and then to carcinoma. In one study although eight of 254 patients had low grade dysplasia,<sup>177</sup> only two of these patients had evidence of dysplasia in subsequent biopsies. Similarly 15 of 22 patients with LGD in another study<sup>167</sup> showed no evidence of dysplasia on repeat biopsy. There may be several explanations for this, including; inaccuracy in the repeat biopsies, a laboratory processing error, or misdiagnosis of, reactive atypia secondary to inflammation and regeneration, which subsequently resolved.

If low grade dysplasia was evident in the rectal cuff, we would recommend six-monthly surveillance biopsies. If these features persisted on three successive biopsies, we would then consider mucosectomy and perineal pouch advancement. Low grade dysplasia should therefore be observed by repeat biopsy.

If cuff biopsies revealed evidence of high grade dysplasia we would recommend multiple biopsies at intervals of 3-6 months and consideration of anal mucosectomy and pouch advancement if these features persist.

Fazio and Tjandra described the technique of transanal mucosectomy being performed to successfully excise retained anorectal mucosa containing foci of high grade dysplasia in a patient that had previously undergone RP for long-standing UC complicated by Dukes C sigmoid carcinoma.<sup>281</sup> They stressed the importance of meticulous dissection to remove a tube of mucosa in its entirety but remarked that mucosectomy and handsewn anastomosis do not prevent rectal cancer in UC pouch patients since rare occurrences of rectal cancer following RP for UC have actually occurred in patients who underwent mucosectomy and IPAA at the time of pouch surgery.<sup>7;52;282</sup> In the former case report, carcinoma was present in the resection specimen but in the latter report this was not the case. This implies that carcinoma in

the pouch, although very rare, is a real complication and should be sought at follow-up by regular clinical examination, endoscopy, CEA levels and transrectal ultrasound where available.

Using dysplasia as the sole marker of cancer risk in cancer surveillance programs is problematic for several reasons; both inter- and intra-observer variability are high, even among experts, agreement on the diagnosis and degree of dysplasia is low, ranging from 42% to, at most, 65%<sup>176;188</sup> and dysplastic cells can be missed in areas of active inflammation, thereby decreasing sensitivity. Carcinoma in UC almost always develops in areas of mucosal dysplasia, unlike sporadic colorectal cancer which develops from adenomatous polyps.<sup>283</sup>

Clearly, the discovery of a pre-dysplastic marker within the columnar cuff would allow the clinician to instigate more frequent surveillance and if necessary, perform a mucosectomy and pouch advancement.

This work presents the first reported figures of p53 immunohistochemical staining of the columnar cuff following RP. Mutation of p53 appears to be an important factor in the tumour biology of colorectal and other neoplasms.<sup>10</sup>

Analysis of the mutations at the molecular level is not currently a routine procedure in pathology laboratories. However, mutant p53 gene product is characterised by a conformational change of the protein with resultant prolonged half-life and stability. The accumulated mutant protein is therefore detectable in nuclei by immunohistochemistry.<sup>10</sup>

We observed no evidence of abnormal stabilisation of p53 and no apparent relationship between p53 stabilisation and dysplasia existed in our study.

When functional, the p53 protein arrests the entry of cells sustaining DNA damage into S phase of the cell cycle, thereby providing an opportunity for DNA repair to proceed through normal repair mechanisms, whereas it triggers apoptosis in cells in which DNA damage is irreversible. Loss of these protective mechanisms through mutation or other means might thereby predispose abnormal epithelia to malignancy, especially under conditions of persistent epithelial injury and turnover, such as those prevailing in UC.<sup>146</sup>

Immunohistochemical overexpression is an indirect manifestation of p53 inactivation. Despite the fact that some p53 point mutations escape detection by immunohistochemistry, they are common in UC-associated dysplasia and cancer. This technique has advantages over molecular DNA assays that make it more suitable for clinical use, including ; technical accessibility, inexpensiveness, accommodation of small tissue samples, and it is associated with less inter-observer variability than dysplasia .<sup>185;197-199;201;206-208</sup> The efficacy of immunohistochemistry may be further enhanced by the development of other antibodies or antibody combinations directed against different p53 epitopes<sup>209</sup> However, there is potential for p53 overexpression to occur in the absence of p53 mutations, as previously reported in non-colorectal tissues, so caution must be applied in interpreting results based solely on this technique. Before p53 loses its function, both a mutation and loss of an allele (loss of heterozygosity) need to occur.. Single strand conformation polymorphism (SSCP) and polymerase chain reaction (PCR) currently are the most precise methods for identifying p53 suppressor gene mutations and loss of heterozygosity but these techniques are currently too expensive and time-consuming for routine use in cancer surveillance.<sup>200</sup>

The immunohistochemical method employed to indicate probable p53 mutation may

have missed some non-sense mutations resulting in a truncated protein. In addition, given the high degree of sensitivity of antigen retrieval methods, overexpression of wild type protein may have been detected i.e. appropriate expression of non-mutant p53 .

Although half our series of cuff biopsies stained positive for p53 overexpression, we cannot conclude that there is apparent abnormal stabilisation of p53 within the columnar cuff, since positive immunohistochemical results were also seen with the negative controls ; normal rectum, normal pouch, inflamed UC and stapled haemorrhoidectomy. It is uncertain why these controls stained positive but it may have been due to the rapid turnover of cells in these distal areas.

Other studies have reported omitting the primary antibody as a negative control but none have described using normal and other forms of intestinal tissue as controls, as in our study, except Taylor et al. who used Pab 1801, a less sensitive monoclonal antibody to p53, and found no positive immunoreactivity in their negative control of normal mucosa from resection margins of lengths of surgically removed colon..

There have been several reports of immunohistochemical positivity in non-colorectal tissues without p53 mutations.<sup>48;210-213</sup> Focussing on colorectal

biopsies, Lashner et al<sup>200</sup> found positive p53 staining in 10/95 patients with chronic UC who had no evidence of dysplasia in their biopsies. They reported that p53 immunohistochemistry was 77% sensitive for dysplasia or cancer and 83% specific for dysplasia or cancer. They concluded that p53 mutations developed approximately eight months before low-grade dysplasia, 26 months before high-grade dysplasia and 38 months before cancer. Harpaz et al. also used immunohistochemistry and found p53 overexpression in 61% of UC-associated colorectal carcinomas, 80% of dysplastic epithelia adjacent to carcinoma and 45% of dysplastic masses remote from



carcinoma<sup>197</sup>. They found independence of p53 overexpression from the grade of dysplasia, suggesting that p53 inactivation in dysplastic epithelia in UC may be an independent marker of malignant potential. Brentnall et al. studied UC-associated neoplastic progression and reported p53 mutations in 29% of biopsies that were negative for dysplasia<sup>199</sup>. They concluded that p53 mutation was an early event in the process of UC-associated tumorigenesis and loss of heterozygosity was a relatively late one. Holzmann et al.<sup>216</sup> used SSCP on UC biopsies to identify p53 mutations in 19% without dysplasia, 41% with indefinite dysplasia, 38% with low grade dysplasia and 63% with high grade dysplasia .

The failure of immunohistochemistry to detect some tumours containing p53 that are identifiable by single strand conformation polymorphism analysis (SSCP) has been noted previously,<sup>214</sup> suggesting that some mutations do not result in detectable overexpression of the p53 protein. Assessment of the value of p53 determination, as an adjunct to conventional histopathological evaluation in the management of high risk UC patients awaits further investigation, including longitudinal studies of patients enrolled in endoscopic surveillance programs. Such studies might ascertain, for example whether p53 status influences the prognostic significance of biopsy specimens with low grade dysplasia.<sup>197</sup>

The presence of aneuploidy in UC in non-dysplastic mucosa is an early and sensitive marker for the development of low-grade dysplasia.<sup>98;205</sup> Aneuploidy often precedes morphologic change by 2-5 years.<sup>205</sup> Melville et al.<sup>98</sup> reported that interobserver variation in assessment of aneuploidy was less than the interobserver variation in the interpretation of dysplasia. Thompson-Fawcett et al. found DNA aneuploidy present in one of their sixty seven patients sampled. Rubin et al.<sup>215</sup> presented a prospective study of 25 high risk patients without dysplasia, among whom 19 without aneuploidy

did not progress to dysplasia in 1-2.5 years but 5 out of 6 patients with aneuploidy developed dysplasia in the same time. Only one patient with aneuploidy did not develop dysplasia. Lofberg et al.<sup>247</sup> reported DNA aneuploidy in a biopsy from the centre of an ileal pouch with low grade dysplasia identified in immediately adjacent tissue.

In larger cohorts that are being surveyed systemically to determine the natural history of the columnar cuff, the DNA content of biopsy specimens would be a useful marker to include, in addition to histology. At present, because cuff neoplasia is uncommon, it seems that little would be gained by routine measurement of DNA content on an individual patient basis. If neoplastic change in the columnar cuff were to become a more frequent problem, flow cytometry for DNA aneuploidy, although arguably useful, still may not gain widespread application in clinical practice, because it has not done so for UC surveillance.

Other markers of pre-dysplastic change in UC have also been studied and E Navratil et al.<sup>32</sup> concluded that further evaluation is needed to determine which additional technologies will, from a practical and epidemiological point of view, optimise the surveillance programme for the rectal mucosa in UC patients who have undergone ileo-rectal anastomosis and might also be useful for long-term surveillance following RP. These additional technologies included; mucin histochemical assessment, loss of heterozygosity for p53 tumour suppressor allele, and DNA aneuploidy detection.

The mucin-associated sialyl-Tn (STn) antigen, as detected by immunohistochemistry with monoclonal antibodies, is another potentially useful marker in this respect and has been shown to precede dysplasia in UC.<sup>44</sup> Karlen et al.<sup>98</sup> reported that STn was frequently expressed in in dysplastic as well as non-dysplastic biopsy specimens from UC patients who developed dysplasia leading to colectomy, but was rarely expressed

in the colorectal mucosa of those who did not develop dysplasia.. They also stated that STn is a marker of cancer risk in UC patients regardless of DNA ploidy status. The potential utility of STn antigen in the clinical treatment of patients with long-standing UC warrants further investigation in prospective studies.

In summary, if p53 testing of surveillance biopsies is combined with testing for dysplasia, two populations of patients with UC will be identified. Patients without dysplasia in the cuff who are p-53 positive could be considered at high risk for the development of carcinoma within the cuff. To reduce cancer mortality, such patients would need to undergo more frequent surveillance testing to detect dysplasia, or to be considered for pouch advancement and mucosectomy before cancer develops.

Likewise, patients with low grade dysplasia but no p53 mutations may be at lower risk for cancer mortality than previously thought and perhaps such patients could delay further surgery until either p53 mutations or high grade dysplasia develop.

In conclusion, we found no evidence of dysplasia or carcinoma within cuff surveillance biopsies . Dysplasia is uncommon in cuff biopsies following RP for UC and has never persisted in the cuff of a patient whose original resection specimen showed no evidence of dysplasia or carcinoma. Whilst positive immunoreactivity is seen within half of cuff surveillance biopsies, no evidence of abnormal stabilisation of p53 could be reported. There was no apparent relationship between p53 stabilisation and dysplasia in these samples.

## **6.2 Pregnancy and sexual function following restorative proctocolectomy**

UC and FAP patients most commonly undergo surgery during their reproductive years when sexual contacts are most frequently established. Lack of information about the potential effects of surgery on sexual function and fecundity has been reported as a major deficiency of pre-operative counselling.<sup>224</sup>

Iatrogenic injury to the autonomic nerves of sexual function is an undisputed complication of proctectomy,<sup>217;225-227</sup> but there is still a paucity of information documenting the resultant sexual dysfunction, specifically for patients who undergo proctectomy as a part of RP. Male sexual dysfunction following RP is has ranged from 2-15% in previous studies. The majority of our patients (88%) were able to achieve orgasm after RP. However we revealed relatively high figures for soiling during intercourse (16%), inability to ejaculate (44%) and erectile dysfunction (32%). We also observed a reduction in sexual frequency in 44% of our patients and reduced quality of orgasm in 35% after RP. The incidence of inability to ejaculate was noticeably high in our series. Whether these results relate to the technical performance of RP in our hands is uncertain. Interestingly, although the longer more laborious technique of close rectal dissection was assumed to be more successful in preserving the autonomic nerves of sexual function, Lindsey et al. found an equal rate of post-operative impotence of 4% whether using this technique or the alternative, total mesorectal excision.<sup>229</sup> It is also encouraging that the majority of male patients with sexual dysfunction following RP, benefited from pharmacological treatment with agents such as sildenafil.<sup>122;128</sup>

Oresland et al<sup>96</sup> showed with radiological hysterosalpinography and vaginography that;10/21 post-RP females had fallopian tubes attached to the bottom of the lesser pelvis, 9/21 had unilateral tubal occlusion and two had bilateral tubal occlusion. They

recommended that the adnexae be protected in temporary peritoneal pouches during surgery. Instillation of bioresorbable membranes has been reported to prevent formation of adhesions,<sup>242</sup> so these substances may have a role to play in improving fertility post-RP. These findings may have contributed to dyspareunia in female patients following RP and such anatomical alterations combined with sphincter dysfunction and damage to the autonomic pelvic plexus may account for the sexual dysfunction seen in this study.

Soiling during intercourse previously reported in 0-15% of females following RP,<sup>96;218;219;222</sup> affected 21% of our female patients and was commonly stated as the reason for reduced intercourse in our female group of patients, which would have an expected impact on fecundity and fertility rates. Special precautions may have to be taken to empty the pouch before intercourse or modifications of sexual technique used to overcome these problems. Clearly, the clinician has a moral responsibility to discuss potential post-operative sequelae such as this with the patient, prior to them consenting to undergo RP. Benefit may be gained from involving a specialist colorectal nurse in these discussions and also from establishing contact with a patient of a similar age group and background who has undergone RP previously.

Vaginal lubrication during intercourse depends on intact pelvic parasympathetic nerve supply, sexual arousal and menopausal status. Neurologically it is the female equivalent of the male erection. We found inadequate lubrication in 46% of our female patients, once again contributing to dyspareunia and subsequent reduction in sexual frequency and pregnancy rates. Interestingly, surgery had no effect upon the ability of female patients to achieve orgasm, 84% could reach climax before and after surgery and this was similar to the percentage of healthy women who have not

undergone surgery<sup>284</sup>. Despite this, 40% of our female patients were dissatisfied with their sex lives post-RP.

43% of women had sex less frequently after surgery, in our study. Whilst half our female patients noted no change in their post-operative libido, 31% reported a reduced libido, compared to 18-25% in other studies.<sup>96;223</sup>

Overall 70% of women were married or co-habiting in stable relationships at the time of the study and whilst one fifth stated that pouch surgery had improved their relationship with their partner, the same percentage (21%) thought that surgery had adversely affected their relationship.

In the literature, several papers that have addressed the issue of fertility and fecundity amongst females undergoing pelvic surgery, including RP.<sup>96;223;233 219;220</sup> However, we are not aware of any reports describing the relationship between RP and male fecundity.

In our series of male patients, fecundity was reduced following RP; pregnancy occurred in the partners of fewer than half of the 13 male patients who were attempting to conceive after RP.

Fecundity refers to the physiological capability of producing a live-born child. In contrast, the term fertility is used to refer to the childbearing performance of a couple and therefore depends upon intention to conceive as well as ability.<sup>220</sup>

In this study, pre-RP *fertility* was 56% therefore pre-operative *fecundity* was at least 56%. It was not possible to quantify accurately the reduction in fecundity compared with the pre-operative status since the proportion of patients who wished to conceive prior to RP was not known.

Although the data demonstrate a relationship between RP and reduced fecundity they do not prove a causative association. Clearly the post-RP results are obtained from a

shorter period of attempted conception with patients being older than they were before pouch formation. The results of postal questionnaire surveys may be confounded by the unrecorded experiences of non-responders that may not mirror those of responders. Furthermore, such retrospective data collection relies on the patients' memory and honesty. Nevertheless, it seems reasonable to attribute reduced fecundity, at least in part, to the documented reduction in libido and sexual frequency after RP. In turn these factors may have been secondary to post-operative sexual dysfunction including soiling during intercourse, impotence and retrograde ejaculation.

This retrospective cohort survey has shown that RP in male patients is associated with a significant incidence of infertility that may be related to post-operative sexual dysfunction. These potential complications should be discussed with patients and their partners prior to RP. Confirmation of the present data by a prospective study with comprehensive follow-up of a larger sample would be an informative exercise that could enhance patient counselling. The merits of pre-emptive measures to assist conception, for example semen storage, might be explored in these patients.

The first report of a successful pregnancy and delivery following RP was in 1984<sup>238</sup> but there is still a paucity of data from large studies concerning this issue<sup>96;230;235;239;240</sup>. Previous studies have documented safe pregnancy and childbirth in patients following RP<sup>219;233;235;238;240;240</sup>. Concerning, the safest method of delivery, caesarean section decreases the risk of incontinence and potential damage to the anal sphincters and pouch but is associated with all the complications of abdominal surgery, including formation of adhesions. Conversely, vaginal delivery may damage the pudendal nerve and anal sphincter mechanism.<sup>239</sup> Some studies have suggested that fertility is unaffected in women following RP,<sup>233;241</sup> but others report an infertility

rate of 20-93% post-RP.<sup>96;230</sup> Post-operative births were only seen in 4-9% of female patients in other large studies<sup>219;239;240</sup> reinforcing the apparent reduction in fertility. Olsen et al.<sup>220</sup> reported 34 post-operative births from 14% of a group of 237 women and this figure represented 49% of the expected number of births in the population. Subtracting those who fell pregnant after IVF, the birth rate fell to 35% of the expected figure. They also revealed 29% of their pregnancies occurred after IVF as opposed to 1% of pregnancies in the normal population.

In our own group of patients the low pregnancy rates post-RP may partly be explained by the reduction in libido and sexual frequency seen in both males and females, which in turn may have been secondary to increased male and female sexual dysfunction post-operatively such as erectile dysfunction, retrograde ejaculation, soiling during intercourse and dyspareunia.

Other than reduced sexual frequency post-RP the other most likely factor contributing to reduced post-operative pregnancy rates is the distortion of vaginal anatomy and effect of adhesions following pelvic surgery. Consideration may be given to delaying pouch-anal anastomosis in patients who have already undergone colectomy and wish to bear children. Specifically, patients with FAP may undergo colectomy and ileo-rectal anastomosis, preserving their nerves of sexual function, until their families are complete. During this period, they will obviously require surveillance for malignant change within the rectum. Subsequently, they may then undergo proctectomy and ileo-anal pouch formation.



We conclude that in our series, pregnancy rates decreased following RP and this was associated with a high incidence of male and female sexual dysfunction. These potential post-operative sequelae and available treatments should be discussed with patients prior to obtaining their consent for surgery.

### **6.3 Haematological outcome following RP**

Vitamin B12 is protein-bound in food and is separated from the protein complex by pepsin in the presence of stomach acid. It is then bound by intrinsic factor and transported from the proximal ileum to the distal ileum, where it is then bound to transcobalamin II for transport to the liver and bone marrow.<sup>244</sup>

These results form the largest reported study with the longest follow-up of haematological results after RP. The results of this study suggest that the risk of developing low serum vitamin B12 levels in patients who have a pouch is much greater than has previously been recognised and that with long-term follow up, one quarter of patients may develop an abnormally low serum B12 following RP. This finding does not appear to be associated with a macrocytic anaemia. The progressive decrease in B12 levels in 73% of patients with multiple B12 readings, suggests that over a longer follow-up period, a much higher percentage of patients may develop vitamin B12 deficiency.

In three smaller series with short follow-up, the incidence of B12 deficiency was reported to be only approximately 5%.<sup>60;61;276</sup> It is assumed that the higher incidence in the present series is related to the longer period of follow up.

The short length of ileal resection and the fact that all but one patient with a sub-normal vitamin B12 had a normal Schilling test in the present study, suggests that removal of ileum was not the cause of the falling B12 levels. The exact minimum length of ileum required for adequate B12 absorption is unknown. Payne and DeWind claim that a 13cm long portion of the distal ileum was sufficient for the absorption of vitamin B12 in adequate amounts,<sup>97</sup> whereas Dano and Lenz suggested 36cm.<sup>193</sup> Thompson et al. concluded that vitamin B12 malabsorption was likely if more than 60cm of terminal ileum was resected.<sup>285</sup> Another group discovered

receptors for the vitamin B12-Intrinsic Factor (B12-IF) complex over, “the entire lower 3/5 of the human small intestine,”<sup>124</sup> supporting the fact that the small amount of terminal ileum excised during RP does not remove the entire area capable of absorbing B12.

Although the exact cause of low vitamin B12 levels in these patients is unknown, it is important to note that in all eight patients given oral vitamin B12, the deficiency was corrected. This suggests that the absorption mechanism is normal and may point to either an inadequate dietary intake or a competitive bacteriological environment for B12 within the pouch. Andres et al. reported that all of their patients with subnormal vitamin B12 levels had normal B12 levels within two months of treatment with oral cyanocobalamin (500-1000µg/day).<sup>6</sup>

We found no evidence of bacterial overgrowth in our B12-deficient patients though previous studies have reported this.<sup>121;253</sup> The abnormal growth of the bacterial flora, possibly associated with changes in the intrinsic factors, the ileal receptor for the intrinsic factor-B12 complex, bacteria utilising vitamin B12, and decreased transit time due to anatomic constraints, might interfere with vitamin B12 absorption.<sup>60</sup> One study demonstrated a reversible absorptive loss of small intestinal capacity in cases of vitamin B12 deficiency and it was suggested that a depletion of vitamin B12 further impairs the absorption of vitamin B12.<sup>60</sup> One study commented that food-cobalamin malabsorption is probably the main cause of vitamin B12 deficiency in adults to i.e. the inability to release vitamin B12 from food or from its binding protein<sup>120</sup>. These patients have low serum vitamin B12 levels, normal Schilling test results and commonly an atrophic gastritis. This syndrome has been reported in association with *Helicobacter pylori* infections.<sup>60;286</sup> It has also been described in association with acid-suppressive drugs<sup>32;146</sup> and metformin use.<sup>130;146</sup>

Although we had a younger study group and none of our B12-deficient patients were taking any of these associated medications, we did not formally perform endoscopy on our patients and check for the presence of atrophic gastritis or *helicobacter pylori* infection, which may have accounted for some of the low vitamin B12 levels.

Vitamin B12 deficiency may correlate may correlate with conditions involving decreased stomach acidity because the protein-bound cobalamin must be separated by acid and pepsin. Since the introduction of H2-receptor antagonists and proton pump inhibitors, the question of possible vitamin B12 malabsorption with these agents has been raised. Swain concluded that long term treatment with 300md/day or more of ranitidine or prolonged treatment with routine doses of omeprazole may cause vitamin B12 deficiency.<sup>287</sup> Our patients with low serum vitamin B12 levels were not taking any of these classes of drug.

In conclusion, we found that at least one quarter of patients will have low serum vitamin B12 levels following RP. This deficiency does not appear to be secondary to either; terminal ileal resection or bacterial overgrowth within the pouch.

Vitamin B12 levels progressively fall in up to two thirds of patients. Since this may have serious clinical consequences, patients should have their B12 levels monitored at regular intervals following pouch surgery and they should be given dietary advice.

This study has also shown that it has been possible to correct low serum vitamin B12 levels in pouch patients by giving oral cyanocobalamin, avoiding the need for injection.

In significant numbers of vitamin B12 deficient patients , serum B12 values fall within the lower end of the normal range.<sup>146,288</sup> Many patients with low vitamin B12 concentrations do not have a clinical syndrome or an underlying disorder compatible with the deficiency .Some authors have found that serum homocysteine and serum

methyl malonic acid (MMA) are two valuable markers for vitamin B12 deficiency.<sup>16;289</sup> As there are only two biochemical pathways that utilise this vitamin in humans, these metabolites may accumulate in the blood stream resulting in elevated levels. Unfortunately, they are far more expensive than testing for serum B12 levels and consequently are not in common use to diagnose vitamin B12 deficiency. Our results form the only reported study on the effect of RP on plasma homocysteine levels. These results indicated that more than one third of pouch patients developed an abnormally elevated homocysteine level following RP. It is accepted that since all these patients had normal serum folate levels, their elevated plasma homocysteine levels may be attributed to vitamin B12 deficiency. Raised homocysteine concentration was infrequently associated with a macrocytic anaemia and no patients developed any of the recognised neurological sequelae of vitamin B12 deficiency, as a consequence. In some cases the macrocytosis may have been counter-balanced by the low ferritin levels seen in 18% of this group, which would favour a microcytic picture.

In our study group, when testing plasma homocysteine levels in all patients with a low serum vitamin B12 reading, elevated results were found in two-thirds.

It is well established that humans have 3-4 years of stores of vitamin B12 if their supplies are completely cut off..<sup>105</sup> The authors' previous work suggests that at least one quarter of patients will develop a low serum vitamin B12 following RP and also identified a progressive decrease in B12 levels with time in 73% of patients who had serial B12 readings taken post RP, suggesting that over a longer follow-up period, a much higher percentage of patients will develop B12 deficiency. Since elevated homocysteine appears to be a more sensitive marker for true low tissue levels of

vitamin B12, the number of patients with a high homocysteine level would be expected to rise considerably above 35% with longer, more frequent assessment. Swain R '95 reported that if both homocysteine and MMA levels are elevated and the serum vitamin B12 level is low or borderline, vitamin B12 deficiency exists. If the homocysteine is the only elevated metabolite and the red blood cell folate level is not low, vitamin B12 deficiency may also be indicated. Since elevated levels of homocysteine may also indicate a folic acid deficiency, red blood cell folate levels should be determined before vitamin B12 replacement is begun. It is important to ensure there is no cobalamin deficiency before starting folic acid replacement, as this treatment may worsen the associated neurological symptoms.

Our results illustrated that all B12 deficient study patients treated on a replacement regimen of 100µg of oral B12 (cyanocobalamin) once a day, had their B12 levels returned to normal after twelve months and this replacement regimen was recommended. The same regimen would be expected to correct elevated plasma homocysteine levels and work is currently in progress to confirm this. A previous study on a different patient group reported that homocysteine levels fall completely into the normal range if B12 replacement is given.<sup>154</sup>

Another homocysteine metabolite whose levels are elevated in vitamin B12 deficiency states, is methyl malonic acid (MMA), because its enzymatic conversion is dependent on a vitamin B12 cofactor. Some studies report that MMA is more sensitive than homocysteine in diagnosing vitamin B12 deficiency<sup>290;291</sup> Savage et al. have shown that elevated levels of MMA are 96-99% accurate as an indicator for cobalamin deficiency.<sup>291</sup> Elevations of MMA are very specific to B12 deficiency because the only other causes likely to be encountered are chronic renal failure or

acute volume depletion.<sup>66;273</sup> Further studies comparing the effect of RP on MMA, homocysteine and vitamin B12 studies are currently underway in our unit.

In conclusion, we found that at least one third of patients will have elevated plasma homocysteine concentrations following RP and with longer follow-up this figure may increase significantly. We recommend for early recognition of treatable vitamin B12 deficiency that patients should have their B12, homocysteine and methyl malonic acid concentrations monitored at regular intervals following pouch surgery and those with abnormal levels should have them successfully restored to a normal value after a year's treatment with oral B12 replacement therapy.

The only other long-term study of haematological outcome following RP was performed by M'koma who reported half the percentage of anaemic patients prior to elective pouch formation (22% compared to 44%). He also reported only 4% of patients were anaemic one year after surgery compared to our 30%. At the same stage 23% of our patients had abnormally low serum ferritin levels. These abnormal results post-RP may be attributable to ; a nutritionally poor Glaswegian diet containing insufficient iron, defective digestion, reduced absorption or continued blood loss- possibly due to "pouchitis" and/or "cuffitis".

M'Koma's results for serum folate reported 14% deficiency in emergency pre-op patients compared to our 25% but he found no abnormal folate levels post-RP in contrast to our findings of 21%. Red cell folate values are more informative since they reflect the folate levels over the previous three months and these were abnormally low in 22% of patients post-RP. Unlike vitamin B12, folate is absorbed in the proximal small bowel and the higher levels of deficiency found in this study may again be explained by poor diet or impaired intestinal absorption.

#### **6.4 Short and long-term nutritional outcome following RP**

Vitamin and trace element deficiency may be due to inadequate dietary intake, decreased absorption, increased requirements and increased losses amongst other reasons.

Ileal epithelium before surgery is predominantly absorptive, with long villi to increase the total surface area in contact with lumen contents.<sup>276</sup> The histological changes in pouch epithelium after RP include; a chronic inflammatory infiltrate in the lamina propria, loss of villous height, crypt hyperplasia and a change in pouch mucin to colorectal type sulfomucin.<sup>276</sup>

Trace elements are absorbed from the small intestine. The metabolism of trace elements is altered in IBD, the most important changes being observed during disease flare-ups.<sup>110;128</sup> Reactive metabolites are produced in excess by the activated phagocytes that infiltrate the inflamed mucosa of IBD patients.<sup>146;292</sup>

The other retrospective study in the literature of nutritional factors following RP<sup>52</sup> did report alterations in some of their parameters after closure of ileostomy including: low zinc levels in up to 5% and low magnesium in up to 32%, although the mean magnesium level steadily rose after ileostomy closure.

They also reported increased IgM levels in 12% of patients at three years and increased IgA levels in 26% eighteen months after ileostomy closure. It is accepted that an increase in IgA is a component of both local and systemic immune response and this would correspond with M'koma et al also reporting that 34% of their study group suffered with pouchitis.<sup>293</sup> A subsequent longer-term study by the same author with a mean of 13.5 years follow-up reported low magnesium levels in 11.3% of patients tested post-RP. Significantly, these patients were suffering with pouchitis at the time of sampling. Hypomagnasaemia is widely reported in chronic diarrhoea,



steatorrhoea and in malabsorption.<sup>293</sup> Long-term results for other trace elements and vitamins were otherwise unremarkable.

Galloway et al. have shown that the presence of a systemic inflammatory response invalidates results for many nutritional parameters,<sup>294</sup> so it may be that the abnormal results seen in this other study, such as low magnesium and zinc levels, were seen in samples taken from patients with pouchitis. They also appeared to include results from patients taking nutritional supplements.

Importantly, Galloway et al. concluded that although vitamins B1, B2 and B12, serum folate and red blood cell levels of glutathione peroxidase are reliable measures of nutritional deficiency, even in the presence of inflammation.<sup>294</sup> Circulating concentrations of many trace elements and commonly measured vitamins are affected by the pathophysiological process of inflammation.. They found that even minor inflammatory episodes with increases in CRP concentrations up to 15mg/L can affect their concentrations.

Kuisma et al.<sup>295</sup> examined the effect of RP on fat-soluble vitamins and vitamin B12 levels. They also included results from patients on vitamin supplements. They reported vitamin A deficiency in 4.8% of post-operative pouch patients vitamin B12 deficiency in 4% and vitamin D deficiency in 10.6%. Otherwise their results for the fat soluble vitamins after ileostomy closure were unremarkable. Vitamin D is normally absorbed in the proximal segments of the small bowel. Tiainen et al also had 4% of their post-operative pouch patients requiring vitamin B12 replacement therapy.<sup>296</sup>

The results of the present study show that on long term follow-up (2-5 and 5-10 years) of patients who have undergone RP, the majority of patients maintain a normal micronutrient profile.

An interesting feature of the present study was that systemic inflammatory response, as evidenced by elevated circulating concentrations of C-reactive protein, was not significantly activated at any of the assessment times of the study. The lack of a systemic inflammatory response is also likely to contribute to the maintenance of a “normal” micronutrient profile.

There have been few long term studies which have examined the nutritional status of patients who had undergone restorative proctocolectomy. The results of the present study are consistent with reports that micronutrient concentrations may recover following successful surgery.<sup>279</sup> Also, more recent studies that suggest that vitamin deficiency is a relatively rare event (<10%) in these patients, even in those followed-up for 4-6 years.<sup>276</sup>

In conclusion, RP appears to be associated with minimal nutritional derangements in the long term after closure of ileostomy.

**Appendix i**    Letter accompanying sexual/reproductive function questionnaire

Dear .....,

At Glasgow Royal Infirmary I look after the 200 patients that have had pouch surgery here.

I understand from your medical records that you have previously undergone surgery for the formation of an ileo-anal pouch and I enclose a questionnaire about various outcomes of your pouch surgery. As you are probably aware this is still a relatively new procedure and a lot has still to be learned about the long term effects of having a pouch formed, hence it is vital that our patients respond to our questionnaire so we can gather as much information as possible.

I fully appreciate that some of these questions are of a very sensitive nature and give you our absolute assurance that your replies will be treated in the strictest confidence and you will remain anonymous from your answers at all times.

If you feel that a particular question is too delicate to answer then please omit it and answer the ones that you are comfortable with, but please try and answer as many as possible.

Your answers will help future patients receive vital information about these important issues before surgery but for the results to be valid, the response rate must be as near 100% as possible.

Yours faithfully,

Mr. D. Coull BSc. MB BS, AFRCS(Ed.)

Surgical Research Fellow

## **Appendix ii** Female sexual/reproductive function questionnaire

What was your diagnosis requiring pouch formation?

Ulcerative Colitis

Familial Polyposis

Slow Transit Constipation

Other (please state)

In which year was the diagnosis made?

In which year was your pouch formed?

Have you ever been given Vitamin B12 injections    Yes/No    If yes please give dates

Have you taken vitamin/mineral/iron or folic acid supplements on a regular basis    Yes/No  
(If yes, what did you take and when did you take it)

Marital Status before pouch operation  
(please circle)

Married, Living with Partner  
Divorced, Separated, Single

Marital Status Now

Married, Living with Partner  
Divorced, Separated, Single

Do you feel that having a pouch formed has had an influence on your long-term relationships  
Yes/No    Please describe

How many times did a wife/partner fall pregnant by you before your pouch operation?

How many children did you father before your pouch operation?

Did your wife/partner try to fall pregnant without success before your pouch operation?    Yes/No  
(i.e. at least 12 months of unprotected sexual intercourse)

Has a wife/partner fallen pregnant by you after your pouch operation?    Yes/No  
If yes how many times?

How many children have you fathered after your pouch operation?

Dates of birth

Has your wife/partner tried to become pregnant without success after your pouch operation?  
(i.e. after at least 12 months of unprotected sexual intercourse)    Yes/No

Have you or your partner/wife been diagnosed with any cause of infertility?  
Please state



### **Appendix iii** Male sexual/reproductive function questionnaire

What was your diagnosis requiring pouch formation?

Ulcerative Colitis

Familial Polyposis

Slow Transit Constipation

Other (please state)

In which year was the diagnosis made?

In which year was your pouch formed?

Marital Status before pouch operation  
(please circle)

Married, Living with Partner  
Divorced, Separated, Single

Marital Status Now

Married, Living with Partner  
Divorced, Separated, Single

Do you feel that having a pouch formed has had an influence on your long-term relationships  
Yes/No Please describe

How many times did a wife/partner fall pregnant by you before your pouch operation?

How many children did you father before your pouch operation?

Did your wife/partner try to fall pregnant without success before your pouch operation? Yes/No  
(i.e. at least 12 months of unprotected sexual intercourse)

Has a wife/partner fallen pregnant by you after your pouch operation? Yes/No  
If yes how many times?

How many children have you fathered after your pouch operation?

Dates of birth

Has your wife/partner tried to become pregnant without success after your pouch operation?  
(i.e. after at least 12 months of unprotected sexual intercourse) Yes/No

Have you or your partner/wife been diagnosed with any cause of infertility?  
Please state

Have you or your partner undergone any fertility treatment(e.g. IVF) – please describe

Were you sexually active before your pouch operation? Yes/No

Have you been sexually active since your pouch operation? Yes/No

How would you describe your sexual interest now compared to before your operation?

**Better**                      **Worse**                      **The Same**

How frequently do you have sexual intercourse now compared to before your operation?

More often                      Less often                      The same

If less often, what do you think is the main reason for this;

- older
- painful intercourse
- reduced libido
- fear of faecal incontinence

- or other reason (please state)

**Do you now suffer with leakage of bowel contents during intercourse? Yes/No**

**Did you suffer with leakage of bowel contents before pouch formation? Yes/No**

Did you have get normal erections before the operation? Yes/No

Have you had difficulty getting erections since the operation? Yes/No

Have you been unable to get an erection since the operation? Yes/No

Were you able to sustain an erection during intercourse before the operation? Yes/No

Have you had problems sustaining an erection after the operation? Yes/No

Did you get painful erections before the operation? Yes/No

Have you suffered with painful erections since the operation? Yes/No

**Were you able to achieve orgasm during intercourse before your operation? Yes/No**

Are you now able to achieve orgasm during intercourse? Yes /No

**Is your ability to achieve orgasm now compared to before the operation?**

The same                      Better                      Worse

Is the quality of the orgasm now , compared to before the operation?

Better                      Worse                      The same

Were you able to ejaculate before the operation ? Yes/No

Have you been unable to ejaculate since the operation? Yes/No

Since your operation have you had a satisfactory sex life? Yes/No  
(if no please state why)

Do you suffer from;diabetes? Yes/No, high blood pressure? Yes/No, vascular disease? Yes/No

**Please state any medication you currently take.**

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